

AEROBIC AND ANAEROBIC DIGESTION OF AGRICULTURAL WASTE FOLLOWED BY VERMICOMPOSTING AND ENRICHMENT

BY

B. PRASANNA KUMAR

B.Sc. (Ag.)

**MASTER OF SCIENCE IN AGRICULTURE
(AGRICULTURAL MICROBIOLOGY)**



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**THESIS SUBMITTED TO THE PROFESSOR JAYASHANKAR
TELANGANA STATE AGRICULTURAL UNIVERSITY IN
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CHAIRPERSON: Dr. S. Triveni



**DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
COLLEGE OF AGRICULTURE
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2016

DECLARATION

I, **B. PRASANNA KUMAR**, hereby declare that the thesis entitled, “**AEROBIC AND ANAEROBIC DIGESTION OF AGRICULTURAL WASTE FOLLOWED BY VERMICOMPOSTING AND ENRICHMENT**” submitted to **Professor Jayashankar Telangana State Agricultural University** for the degree of **MASTER OF SCIENCE IN AGRICULTURE** in the major field of Agricultural Microbiology, is the result of original research work done by me. I also declare that no material contained in the thesis or any part thereof has not been published earlier in any manner.

Place: Hyderabad

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Date:

I. D. No. RAM/2014-81

CERTIFICATE

This is to certify that the thesis entitled “**AEROBIC AND ANAEROBIC DIGESTION OF AGRICULTURAL WASTE FOLLOWED BY VERMICOMPOSTING AND ENRICHMENT**” submitted in partial fulfillment of the requirements for the degree of “**MASTER OF SCIENCE IN AGRICUTLURE**” of the **Professor Jayashankar Telangana State Agricultural University, Hyderabad**, is a record of the bonafide research work carried out by **Mr. B. Prasanna Kumar** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigation have been duly acknowledged by the author of the thesis.

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LIST OF CONTENTS

Chapter No.	Title	Page No.
I	INTRODUCTION	
II	REVIEW OF LITERATURE	
III	MATERIAL AND METHODS	
IV	RESULTS AND DISCUSSION	
V	SUMMARY AND CONCLUSIONS	
	LITERATURE CITED	
	APPENDICES	

LIST OF TABLES

Table No.	Title	Page No.
3.6.1	Details of the treatments	
3.6.2	Details of the treatments	
4.1	Isolation of cellulose degrading bacteria	
4.1.1.A	Morphological characteristics of cellulose degrading bacteria	
4.1.1.B	Biochemical characteristics of cellulose degrading bacterial isolates	
4.1.2	Cellulase activity of bacterial isolates	
4.1.3	Cellulase activity of bacterial isolates	
4.2	Chemical composition of different substrates at initial stage used in the treatments of Agricultural waste	
4.3	Population of bacteria, fungi and actinomycetes in agricultural waste	
4.4	Total solids (%), total volatile solids (%) and volatile fatty acids content in agricultural waste at different intervals.	
4.5	pH in different treatments with pretreatment of agriculture waste	
4.6	Nitrogen, Phosphorus and Potassium content in different treatments with pretreatment of agricultural waste at different intervals	
4.7	Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments on pretreatmet of agricultural waste at different intervals.	
4.8	Cellulose content in different treatments as pretreatment of agriculture waste	
4.9	Population of bacteria, fungi and actinomycetes in different pretreated substrates of agricultural waste	
4.10	Total solids (%), total volatile solids (%) and volatile fatty acids content in aerobic composting and anaerobic digestion of agricultural waste at different intervals.	

4.11	pH in the slurry samples	
4.12	Nitrogen, Phosphorus and Potassium content in different treatments during aerobic composting and anaerobic digestion of agricultural waste at different intervals.	
4.13	Contents of Organic carbon, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) in different treatments during aerobic composting and anaerobic digestion of agricultural waste at different intervals.	
4.14	Cellulose content in different treatments in agricultural waste	
4.15	Population of bacteria, fungi and actinomycetes during aerobic composting and anaerobic digestion of agricultural waste	

Table No.	Title	Page No.
5.1	Chemical composition of different substrates at initial stage used in the treatments of Horticultural waste	
5.2	Population of bacteria, fungi and actinomycetes in horticultural waste	
5.3	Total solids (%), Total Volatile Solids (%) and Volatile Fatty Acids in horticultural waste at different intervals.	
5.4	pH in different treatments with pretreatment of horticultural waste	
5.5	Nitrogen, Phosphorus and Potassium content in different treatments with pretreatment of horticultural waste at different intervals	
5.6	Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments as pretreatment of horticultural waste at different intervals.	
5.7	Cellulose content in different treatments as pretreatment of horticultural waste	
5.8	Population of bacteria, fungi and actinomycetes in different pretreated substrates of horticultural waste	

5.9	Total Solids (%), Total Volatile Solids and Volatile Fatty Acids content in aerobic composting and anaerobic digestion of horticultural waste at different intervals	
5.10	pH in the slurry samples	
5.11	Nitrogen, Phosphorus and Potassium content in different treatments during aerobic composting and anaerobic digestion of horticultural waste at different intervals	
5.12	Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments during aerobic composting and anaerobic digestion of horticultural waste at different intervals	
5.13	Cellulose content in different treatments in horticultural waste	
5.14	Population of bacteria, fungi and actinomycetes in different substrates of horticultural waste	
5.15	Production of biogas from agricultural and horticultural waste	
5.16	Bioethanol production from agricultural and horticultural waste	

LIST OF ILLUSTRATIONS

Illustration No.	Title	Page No.
4.1	Contents of Total solids (%), total volatile solids (%) and volatile fatty acids in agricultural waste with pretreatment at different intervals.	
4.2	pH in different treatments with pretreatment of agriculture waste	
4.3	Nitrogen, Phosphorus and Potassium content in different treatments with pretreatment of agricultural waste at different intervals	
4.4	Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments on pretreatmet of agricultural waste at different intervals.	
4.5	Cellulose content in different treatments with pretreatment of agriculture waste	
4.6	Population of bacteria, fungi and actinomycetes in different pretreated substrates of agricultural waste	
4.7	Total solids (%), total volatile solids (%) and volatile fatty acids in aerobic composting and anaerobic digestion of agricultural waste at different intervals.	
4.8	pH in the slurry samples	
4.9	Nitrogen, Phosphorus and Potassium content in different treatments during aerobic composting and anaerobic digestion of agricultural waste at different intervals.	
4.10	Contents of Organic carbon, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) in different treatments during aerobic composting and anaerobic digestion of agricultural waste at different intervals.	
4.11	Cellulose content in different treatments in agricultural waste	
4.12	Population of bacteria, fungi and actinomycetes during degradation of agricultural waste	

Illustration No.	Title	Page No.
5.1	Contents of Total Solids (%), Total Volatile Solids (%) and Volatile Fatty Acids in horticultural waste with pretreatment at different intervals.	
5.2	pH in different treatments with pretreatment of horticultural waste	
5.3	Nitrogen, Phosphorus and Potassium content in different treatments with pretreatment of horticultural waste at different intervals	
5.4	Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments as pretreatment of horticultural waste at different intervals	
5.5	Cellulose content in different treatments with pretreatment of agriculture waste	
5.6	Population of bacteria, fungi and actinomycetes in different pretreated substrates of horticultural waste	
5.7	Total Solids (%), Total Volatile Solids and Volatile Fatty Acids in aerobic composting and anaerobic digestion of horticultural waste at different intervals	
5.8	pH in the slurry samples	
5.9	Nitrogen, Phosphorus and Potassium content in different treatments during aerobic composting and anaerobic digestion of horticultural waste at different intervals	
5.10	Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments during aerobic composting and anaerobic digestion of horticultural waste at different intervals	
5.11	Cellulose content in different treatments in horticultural waste	
5.12	Population of bacteria, fungi and actinomycetes in different substrates of horticultural waste	
5.13	Production of biogas from agricultural and horticultural waste	

5.14	Bioethanol production from agricultural and horticultural waste	
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LIST OF PLATES

Plate No	Title	Page No.
4.1	Isolation of cellulose degrading bacterial isolates	
4.2	Screening of cellulose degrading bacterial isolates	
4.3	Biochemical characters of the cellulose degrading isolates	
4.4	Hot plate with samples kept for digestion	

LIST OF APPENDICES

Appendix No.	Title	Page No.
I	Composition of different growth media/ reagents/ indicators used	
2	Reagents used for chemical analysis of substrates used for composting, vermicomposting, bioethanol and biogas production	

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Place:

(B. Prasanna Kumar)

Date:

LIST OF SYMBOLS AND ABBREVIATIONS

Cm	:	Centimetre
&	:	And
M	:	Metre
G	:	Gram
Kg	:	Kilogram
T	:	Tonn
Ha	:	Hectare
kg ha ⁻¹	:	Kilogram per hectare
t ha ⁻¹	:	Tonn per hectare
Kmph	:	Kilometre per hour
dS m ⁻¹	:	Decisiemen per metre
@	:	At the rate of
SEm	:	Standard error of mean
CD(P=0.05)	:	Critical difference at 5 percent level
<i>et al.</i>	:	And others
H	:	Hours
%	:	Per cent
NS	:	Non significant
°C	:	Degree Celsius
N	:	Nitrogen
P	:	Phosphorus

K	:	Potassium
OC	:	Organic carbon
pH	:	Pussancea hydrogen
viz.,	:	Namely
μ	:	Microns
Rs	:	Rupees
g cc ⁻³	:	Gram per cubic centimetre
number m ⁻²	:	Number per square metre
SSP	:	Single super phosphate
MOP	:	Muriate of potash
Max	:	Maximum
Min	:	Minimum
day ⁻¹	:	Per day
Mm	:	Millimetre
RDF	:	Recommended dose of fertilizers
FYM	:	Farmyard manure
Fig.	:	Figure
hour ⁻¹	:	Per hour
Ppm	:	Parts per million
ml lit ⁻¹	:	Millilitre per litre
cm ²	:	Square centimetre
NO ₃ ⁻	:	Nitrate

RDF	:	Recommended dose of fertilizer
No.	:	Number
vis-à-vis	:	In relation to
F- test	:	Fisher's test
AW	:	Agricultural waste
HW	:	Horticultural waste
OKW	:	Organic kitchen waste
CDS	:	Cow dung sample
MSW	:	Municipal solid waste
DKW	:	Domestic kitchen waste
FF	:	Farmers' fields
HDD	:	Horse dung dump
FPC	:	Filter paper cellulase
TMS	:	Total mass of slurry
FVW	:	Fruit and vegetable waste
PGPM	:	Plant growth promoting microbes
GHGs	:	Greenhouse gases
CO ₂	:	Carbon dioxide
CMC	:	Carboxy methyl cellulose
CDB	:	Cellulose degrading bacteria
CFU	:	Colony forming unit
VC	:	Vermicompost
CD	:	Cow dung
TKN	:	Total kjedahl nitrogen
MSW	:	Municipal solid waste
TS	:	Total solids
TVS	:	Total volatile solids
VFA	:	Volatile Fatty Acids
BOD	:	Biological Oxygen Demand
D.O	:	Dissolved Oxygen
D.O ₁	:	Dissolved Oxygen on the first day
D.O ₅	:	Dissolved Oxygen Demand on the

	:	fifth day
COD	:	Chemical Oxygen Demand
M	:	Cattle Manure
Spp.	:	Species
CRD	:	Completely Randomized Design
R ₁	:	Replication 1
R ₂	:	Replication 2
R ₃	:	Replication 3
NA	:	Nutrient agar
SPAD	:	Soil plant analytical development
Mg	:	Milligram
L	:	Litre
CH ₄	:	Methane
	:	
IAA	:	Indole acetic acid
NaOH	:	Sodium Hydroxide
HCL	:	Hydrochloric acid
H ₂ SO ₄	:	Sulphuric acid
APHA	:	American Public Health Association
K ₂ Cr ₂ O ₇	:	Potassium dichromate
°	:	degrees
'	:	minutes
"	:	seconds

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ABSTRACT

In the present study the experiment was conducted during 2015-16 at Department of Agricultural microbiology and Bioenergy, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad.

To expand the range of natural bio-resources the rapidly evolving tools of biotechnology can lower the conversion costs and also enhance target yield of the product of interest. Cellulolytic microorganisms have been isolated from different soil samples and also from waste samples characterized and screened for their efficiency in cellulose degrading activity. The best isolates were further tested for their enzyme activity. These efficient isolates were used for the pretreatment of agricultural waste (Maize straw) and horticultural waste (Banana fruit waste) prior to aerobic and anaerobic digestion determination. Cowdung along with agricultural waste and horticultural waste were used for composting, vermicomposting, biogas production and bioethanol production in lab scale. By the end of the process of aerobic composting and anaerobic digestion of agricultural waste the reduction percentage of Total Solids (TS) (14.99 %), pH (7.58), Chemical Oxygen Demand (COD) (190.82 g l⁻¹), Cellulose (16.20 %), bacterial population count (6.4 x 10⁶ CFU g⁻¹) and actinomycetes (6.1 x 10⁴ CFU g⁻¹) was significantly more in T₁ (Composting without pretreatment). Reduction of Organic carbon (31.32 %) was significantly more in T₂ (Composting with pretreatment). Reduction in Total Volatile Solids (TVS) (76.48 %), Nitrogen (2.00 %) and Potassium (1.50 %) was significantly more in T₃ (Vermicomposting without pretreatment). Reduction of Volatile Fatty Acids (VFA) (0.38 g l⁻¹) was significantly more in T₄ (Vermicomposting with pretreatment). Change in pH (7.58) was significantly more in T₅ (Biogas production without pretreatment). Reduction in Phosphorus (1.65 %), Biological Oxygen Demand (BOD) (90.65 mg l⁻¹) and fungal population count (3.8 x 10⁴ CFU g⁻¹) was significantly more in T₆ (Biogas production with pretreatment). By the end of the process of aerobic composting and anaerobic digestion of agricultural waste the deterioration of Nitrogen (1.05 %), Potassium (0.68 %), Biological Oxygen Demand (BOD) (45.80 mg l⁻¹) and fungal population count (2.1 x 10⁴ CFU g⁻¹) was significantly less in T₁ (Composting without pretreatment). Reduction in Phosphorus (0.38 %) was significantly less in T₂ (Composting with pretreatment). Reduction in Organic carbon

(22.52 %) and pH (7.21) and bacterial population count (3.5×10^6 CFU g⁻¹) was significantly less in T₄ (Vermicomposting with pretreatment). Reduction in Total solids (TS) (7.05 %), Total volatile solids (TVS) (45.29 %) and Chemical Oxygen Demand (COD) (59.46 g l⁻¹) was significantly less in T₅ (Biogas production without pretreatment). Reduction in Volatile fatty acids (VFA) (2.26 g l⁻¹) and cellulose (12.85 per cent) and actinomycetes (4×10^4 CFU g⁻¹) was significantly less in T₆ (Biogas production with pretreatment).

Similarly in horticultural waste by the end of the process of aerobic composting and anaerobic digestion the change in pH (7.90) and actinomycetes (7.2×10^4 CFU g⁻¹) was significantly more in T₁ (Composting without pretreatment). Reduction in Total Solids (TS) (16.15 %) and Chemical Oxygen Demand (COD) (96.25 g l⁻¹) was significantly more in T₂ (Composting with pretreatment). Reduction in Total Volatile Solids (TVS) (78.72 %), Biological Oxygen Demand (BOD) (85.15 mg l⁻¹) and cellulose (17.36 per cent) was significantly more in T₃ (Vermicomposting without pretreatment). Reduction in Phosphorus (1.58 %) and Potassium (1.56 %) was significantly more in T₄ (Vermicomposting with pretreatment). Reduction in Organic carbon (45.7 %) and fungi (11×10^4 CFU g⁻¹) was significantly more in T₅ (biogas production without pretreatment). Reduction in Volatile Fatty Acids (VFA) (1.25 g l⁻¹) Nitrogen (2.30 %) and bacteria (9.8×10^6 CFU g⁻¹) was significantly more in T₆ (Biogas production with pretreatment). By the end of the process of aerobic composting and anaerobic digestion of horticultural waste the deterioration of fungi (7×10^4 CFU g⁻¹) was significantly less in T₁ (Composting without pretreatment). The reduction in Nitrogen (1.30 %), Phosphorus (0.31 %), Potassium (0.60 %), Organic carbon (23.2 %) and bacteria (5.7×10^6 CFU g⁻¹) was significantly less in T₂ (Composting with pretreatment). Reduction in Volatile Fatty Acids (0.60 g l⁻¹) was significantly less in T₃ (Vermicomposting without pretreatment). Change in pH (7.33) was significantly less in T₄ (Vermicomposting with pretreatment). Reduction in Total solids (TS) (6.99 %), Total volatile solids (TVS) (53.41 %), Biological Oxygen Demand (BOD) (49.45 mg l⁻¹) and Chemical Oxygen Demand (COD) (59.39 g l⁻¹) was significantly less in T₅ (Biogas production without pretreatment). Reduction in cellulose (14.03 %) and actinomycetes (3.7×10^4 CFU g⁻¹) was significantly less in T₆ (Biogas production with pretreatment).

At the end of the 60th day the methane gas production was significantly more in AW-T₂ (Biogas production with pretreatment) 3531.10 ml, compared to AW-T₁ (Biogas production without pretreatment) 3381.00 ml, HW-T₂ (Biogas production with pretreatment) 2620.70 ml and less in HW-T₁ (Biogas production without pretreatment) 2381.40 ml. The content of bioethanol was less in HW-T₁ (Bioethanol production without pretreatment) 0.050 g L⁻¹ compared to less in HW-T₂ (Bioethanol production with pretreatment) 0.047 g l⁻¹, AW-T₂ (Bioethanol production with pretreatment) 0.036 g l⁻¹ and less in AW-T₁ (Bioethanol production without pretreatment) 0.034 g l⁻¹. In this present study results revealed agricultural and horticultural waste pretreatment with efficient microbes played good role during aerobic composting, vermicomposting, and anaerobic bioethanol and biogas production capabilities. Hence, composting, vermicomposting, biogas production has proved to be effective and efficient in conversion of wastes into value added products. Hence it can be concluded that horticultural waste was comparatively better in N, P, K and Organic carbon % composition while agricultural waste was good for compost and vermicompost making and also useful for the production of biogas.

Chapter I

INTRODUCTION

Agricultural crop residues are generated in large quantities and constitute an abundant but underutilized source of renewable biomass in agriculture. The amount of crop residues available in India is estimated to be approximately 620 million tons. Half the quantity of agro-residues thus produced finds use as roofing material, animal feed, fuel and packing material, while the other half is disposed of by burning in the field. Burning agro-residues in the field is considered a cheap and easy means of disposal of excess residues. Direct incorporation of agro-residues like rice straw in field solves the problem of air pollution but it is not feasible due to the short time gap between harvesting. Moreover, observations of long term experiments indicate that through incorporation of agro-residues in soil improves soil health significantly, paddy straw increases the methane emission from field especially in irrigated soils, which in turn adds to the malice of global warming which decreases the subsequent crop yields due to production of allelochemicals and immobilization of the available nitrogen (Singh and Nain, 2014). Fruit and Vegetable Wastes (FVW) are produced in large quantities in markets, and constitute a source of nuisance in municipal landfills because of their high biodegradability. In India, FVW constitute about 5.6 million tonnes annually and currently these wastes are disposed by dumping on the outskirts of cities (Bouallagui *et al.*, 2005).

However, plant biomass considered as “waste” can potentially be converted into various different value added products including biofuels, chemicals, animal feed, textile, laundry, pulp, paper, composting, vermicomposting and production of ethanol from lignocellulosic biomass can reduce air pollution, decrease the release of carbon dioxide in the atmosphere, and provide new markets for agricultural wastes (Manmeet Kaur and Arora, 2012).

Biodegradation is breakdown of organic contaminants occurring due to production of extracellular enzymes by microorganisms. These contaminants can be considered as the substrate or microbial food source. These large volumes of cellulosic waste generated are difficult to degrade and cause imbalances in the ecosystem. All these processes contribute to pollution of water with highly turbid greyish water and mercaptan like smell that pervades the yards and nearby areas. This is reported to have a devastating effect on flora and fauna (Shenkani and Sundara, 2015). The pretreatment is to make the

lignin, cellulose and hemicellulose of waste material into small molecules by cellulose degrading microorganisms. Compared to chemical pretreatment microbial pretreatment has great potential on the degradation of complex hydrolytic enzymes into smaller sugar components like glucose units. A large number of cellulolytic bacteria (*Bacillus licheniformis*, *Pseudomonas* sp., *Bacillus subtilis*, and *Pleurotus florida*) and fungi have been isolated by various investigators from self-heating material and other sources play an important role in decomposing of municipal solid waste. Fungi capable of producing extracellular enzymes responsible for degradation of cellulose are known, some of them being highly cellulolytic, which include species of *Aspergillus* sp., *Trichoderma* sp., *Sclerotium* sp. and are also being considered for commercial exploitation (Gautam *et al.*, 2010).

Methane, which is the main component of biogas, is a valuable renewable energy source, but also a harmful greenhouse gas if emitted into the atmosphere. Methane, upgraded from biogas, can be used for heat and electricity production or as biofuel for vehicles to reduce environmental emissions and the use of fossil fuels. The anaerobic digestion process is a natural biological process in which a community of bacteria cooperates to form a stable, self-regulating fermentation that converts waste organic matter into a mixture of carbon dioxide and methane gases. In biogas manure digester bacterial community generally operates as three interdependent groups: hydrolytic bacteria, acid-forming bacteria and methanogenic bacteria. Composting is the most economical and sustainable option for organic waste management. Composting is a natural process of organic waste treatment which is currently practised with various modifications to the technology. Composting using worms, known as vermicomposting gives a better end product (vermicastings) than composting due to the enzymatic and microbial activity that occur during the process. (Jaya *et al.*, 2006).

Combustion of fossil fuels has released lot of greenhouse gases (GHGs), which contain high amount of carbon di-oxide (CO₂) and methane gas (CH₄). The effective alternative for these constraints is the utilization of bio-mass as renewable source & production of ethanol from carbohydrate (bio-mass) (Balasubramanian *et al.*, 2011). Bioethanol is regarded as a promising alternative energy source, which is both renewable and environment friendly (Veeranjaneya *et al.*, 2014). In India, sugar cane molasses is the main raw material for ethanol production. But the short supply and increased cost is the main hindrance for its use. The cellulosic materials are cheaper and available in plenty but their conversion to ethanol involves many steps and is therefore expensive. Under

such circumstances a novel approach is essential to use renewable substrates such as fruit waste. In this respect, inexpensive raw materials such as agricultural wastes, cellulosic wastes, fruit wastes, vegetable wastes, municipal and industrial wastes can be used to produce ethanol cheaply. Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology (Ajay *et al.*, 2014).

Vermicompost is nutritionally rich natural organic fertilizer involving in the joint action of earthworms and microorganisms, which releases nutrients relatively slowly in the soil and improves quality of the plants along with physical and biological properties of soil. It has a more beneficial impact on plants than soil. These composts provide all nutrients in readily available forms and also enhances uptake of nutrients by plants and plays a major role in improving growth and yield of different field crops (Khan and Ishaq, 2011). The epigeic earthworms were utilized for organic wastes management. During vermicomposting the nutrients are released and converted into soluble and available forms to plants. The earthworms can tolerate temperatures ranging from 0 °C to 40 °C with pH of 7 but the regeneration capacity is more at 25 °C to 40 °C and 40–45 % moisture content and partially decomposed organic matter is rich in nitrogen. Crop residues were recycled through vermicomposting by using the epigeic earthworm *Eudrillus eugeniae*. It has been well established that epigeic forms of earthworms can hasten the composting process to a significant extent with production of better availability of vermicomposts. The epigeic earthworm *E. fetida* is a suitable species for management of wastes which are utilized successfully in vermicomposting (Gorakhnath *et al.*, 2009).

Objectives of investigation

- 1) Isolation of cellulolytic microbial consortium and pre-treatment of agricultural and horticultural wastes
- 2) Aerobic composting and anaerobic digestion of agricultural and horticultural wastes
- 3) Methane percentage will be measured with the help of Gas chromatography, Alcohol estimation by colorimeter / Gas chromatography.

Chapter II

REVIEW OF LITERATURE

Waste management is the collection, transport, processing, recycling or disposal, and monitoring of waste materials. Concern over environment is being seen a massive increase in recycling globally which has grown to be an important part of modern civilization. Waste generation per capita has increased and is expected to continue to climb with growing population, wealth and consumerism throughout the world. Approaches to solving this waste problem in a scalable and sustainable manner would lead us to a model that uses waste as an input in the production of commodities and value monetized, making waste management a true profit centre.

The conversion of waste as a potential source of energy has a value as a supplemental feedstock for the rapidly developing bio-fuels sector. Thermal technologies like gasification, pyrolysis, thermal depolymerisation and non-thermal technologies like anaerobic digestion, fermentation are a number of new and emerging technologies that are able to produce energy from waste and other fuels without direct combustion. Biodegradable wastes are processed by composting, vermicomposting, anaerobic digestion or any other appropriate biological processing for the stabilization of wastes. There is a clear need for the current approach of waste disposal in India that is focussed on municipalities and uses high energy / high technology, to move more towards waste processing and waste recycling (that involves public-private partnerships, aiming for eventual waste minimization driven at the community level and using low energy / low technology resources).

The relevant literature reviewed pertaining to the present study has been summarized and presented in this chapter.

2.1. ISOLATION OF CELLULOLYTIC MICROBIAL CONSORTIUM AND PRE-TREATMENT OF AGRICULTURAL AND HORTICULTURAL WASTES

2.1.1 Sample Collection and Identification of Cellulolytic Microbial Organisms

Ashwani *et al.* (2014) focused on the isolation of efficient cellulolytic bacteria found in forest soil rich with plant residues. The test soil with plant residues was assessed for physico chemical and biological properties and the presence of plant residues in the

soil caused changes in physico chemical & biological properties of soil. These changes include increase in clay and silt percentages, electrical conductivity, water holding capacity, organic matter, total nitrogen, available phosphorus and potassium content in test over control. However there was a less sand, higher bacterial and fungal populations were recorded in test soil. Cellulose degrading bacteria were isolated from the forest soil rich with plant residues by using cellulose congo red agar medium. The isolates were morphologically and biochemically characterized and pure isolates were screened for cellulase activity.

Koteswararao *et al.* (2014) reported the use of cellulose containing soil as a source to isolate the cellulolytic bacteria. Initial isolation was performed using congo red agar medium. Out of the 15 colonies observed one of the most prominent colonies was collected and purified by streak plate method. Degradation zones were identified on the CMC medium and were denoted as R-1. Morphological and bio-chemical tests confirmed the organism as *Bacillus* sp., which was grown on different cellulose media as CMC, cellulose powder and cellobiose for different incubation times. Effect of incubation time for the above mentioned substrates on cellulolytic activity was studied by comparing diameters of hydrolysis zones, which are considered as a measure of cellulolytic efficiency. Maximum cellulolytic activity was observed in cellobiose substrate at 0.2 % concentration on 12th day of incubation.

Lekh ram *et al.* (2014) reported that the cellulose can be hydrolysed by using enzymes to produce glucose, which can be used for the production of ethanol, organic acids and other chemicals. This enzyme has enormous potential in industries and can be used in food, beverages, textile, laundry, paper and pulp industries etc. Therefore, there has been much research aimed at obtaining new microorganisms producing cellulose enzymes with higher specific activities and greater efficiency. 21 fungal strains were isolated from the soil samples, out of which four isolates were showing the cellulase activity this was assayed by Carboxymethyl cellulase “CMCase” (endoglucanase) assay.

Meghdad *et al.* (2013) used a plastic container which was prepared for vermicomposting, then bed was prepared in a ready container with a layer of initial bedding, sieved garden soil and compostable waste. It was inoculated with *Eisenia fetida* earthworms. They concluded that vermicomposting was the best and most suitable method for solid waste disposal.

Shaikh *et al.* (2013) isolated that the cellulase producing bacteria from various region including paper industry waste, municipal waste, sugarcane farm, garden, and wood furnishing region. Total 34 isolates were obtained by the primary screening technique from which 11 isolates were showing maximum cellulase activity. Potential isolates were obtained from wood furnishing region and paper industry waste. These 11 isolates were then evaluated by secondary screening for enzyme production. Among these 11 isolates CDB-27 and CDB-30 were selected as most efficient enzyme producers and their specific enzyme activity in the crude sample. Isolates were tentatively characterized on the basis of their cultural and morphological and biochemical characteristics, CDB-27 and CDB-30 were identified to be *Pseudomonas sp.* and *Bacillus sp.* respectively. Optimization of different parameters was carried out for the production of cellulase by both efficient isolates. The maximum enzyme producing isolate CDB-30 was used further to check biodegradation properties at laboratory scale.

Gautam *et al.* (2012) reported that the municipal solid waste contains high amounts of cellulose, which is an ideal organic waste for the growth of most of microorganism as well as composting by potential microbes. Congo red test was performed for screening of microorganism after selecting potential strains. It was further used for biodegradation of organic municipal solid waste. Forty nine out of the 250 different microbes tested (165 belong to fungi and 85 to bacteria) produced cellulase enzyme and among these *Trichoderma viride* was found to be a potential strain in the secondary screening. During the biodegradation of organic waste, after 60 days, the average weight losses were 20.10 % in the plates and 33.35 % in the piles. There was an increase in pH until 20 days. pH however, stabilized after 30 days in the piles. Temperature also stabilized as the composting process progressed in the piles. The high temperature continued until 30 days of decomposition, after which the temperature dropped to 40 °C and below during the maturation. Good quality compost was obtained in 60 days.

Pratima *et al.* (2012) isolated that the cellulose-degrading bacteria (CDB) were isolated by enriching the basal culture medium with filter paper as substrate for cellulose degradation. To indicate the cellulose activity of the organisms, diameter of clear zone around the colony and hydrolytic value on cellulose Congo red agar media were measured. CDB-8 and CDB-10 exhibited the maximum zone of clearance around the colony with diameter of 45 and 50 mm and with the hydrolytic value of 9.0 and 9.8, respectively. The enzyme assays for two enzymes, Filter Paper Cellulase (FPC) and

cellulose (endoglucanase) were examined by methods recommended by the International Union of Pure and Applied Chemistry (IUPAC). The maximum filter paper degradation percent was estimated to be 65.7 for CDB-8.

Barman *et al.* (2011) conducted the experiment to find out the effective cellulolytic bacteria for biodegradation of solid kitchen and agricultural wastes as organic manure or compost. Bacterial strains of *Moraxella* sp. *Cellulomonas* sp. and *Planococcus* sp. were isolated from soil and cultured on nutrient agar media. In comparison to *Cellulomonas* sp. and *Planococcus* sp. inoculation of *Moraxella* sp. enhanced the degradation of kitchen and agricultural waste, shown by the increased CO₂ release (54.3 and 37.62 mg), crude fiber loss (46.86 % and 45.11 %), total sugar reduction (72.52 % and 74.27 %), fat reduction (65.20 % and 61.22 %), endoglucanase (0.097 mg. hr⁻¹. ml⁻¹) and cellobiase (0.82 mg. hr⁻¹. ml⁻¹) activities. Inoculation of *Cellulomonas* sp. strain (53.89 % and 77.96 %) showed high protein reduction in comparison with inoculation of *Moraxella* sp. strain (20.04 % and 63.42 %) for kitchen and agricultural wastes. The overall findings of this investigation demonstrate that the effectiveness of *Moraxella* sp. as a useful strain for bioconversion of solid organic waste.

Ramesh *et al.* (2008) evaluated the screening for cellulase-producing microorganisms is routinely done on carboxy methyl cellulose (CMC) plates. The culture plates are flooded either with 1 % hexadecyl trimethyl ammonium bromide or with 0.1 % congo red followed by 1 M NaCl. An improved method was reported for the detection of extracellular cellulase production by microorganisms by way of plate assay. In this method, CMC plates were flooded with Gram's iodine instead of the reagents just mentioned. Gram's iodine formed a bluish black complex with cellulose but not with hydrolysed cellulose, giving a sharp and distinct zone around the cellulose producing microbial colonies within 3 to 5 minutes. The new method is rapid and efficient; therefore, it can be easily performed for screening large numbers of microbial cultures of both bacteria and fungi. This is the first report on the use of Gram's iodine for the detection of cellulose production by microorganisms using plate assay.

2.1.2 Biological Pretreatment of Agricultural and Horticultural Wastes

Shruti *et al.* (2015) reported that the lignocellulose containing plants are those types of biomass that include wood, agricultural residues and paper wastes. They are composite polymeric material containing cellulose, hemicellulose and lignin. Rice straw degrading microbial consortium was isolated. Nineteen microorganisms were isolated,

among 14 were bacterial strains and four were fungal strains. The isolated bacterial strains were characterized using gram staining and biochemical tests. Four isolated fungal species were characterized by lactophenol cotton blue staining. Three of the isolated fungal strains were identified as *Aspergillus* and one of them was identified as *Gliocladium*.

Shuxia *et al.* (2012) reported that stack-pretreatment was introduced to gain higher biogas production from corn stover through solid state anaerobic digestion. The corn straw had been stack pretreated for 20 days, to decrease the content of the middle corn straw cellulose, hemi-cellulose and lignin by 5.8 %, 16.8 % and 5.7 % respectively. The result indicated that the biogas yield of anaerobic digestion of the pretreated corn stover mixed with cow dung is higher than that of the untreated ones mixed with cow dung, and higher than that of the untreated ones alone, also higher than the cow dung alone. The biogas yield of the pretreated corn stover mixed with cowdung is higher than the pretreated ones with the sludge.

Weizhang *et al.* (2011) conducted an experiment that the biological pre-treatment with new complex microbial agents were used to pre-treat corn straw at ambient temperature (about 20 °C) to improve its biodegradability and anaerobic biogas production. A complex microbial agent dose of 0.01 % (w/w) and pre-treatment time of 15 days were appropriate for biological pre-treatment. These treatment conditions resulted in 33.07 % more total biogas yield, 75.57 % more methane yield and 34.6 % shorter technical digestion time compared with the untreated sample. These changes contributed to the enhancement of biogas production. Biological pre-treatment could be an effective method for improving biodegradability and enhancing the highly efficient biological conversion of corn straw into bioenergy.

Fred *et al.* (2003) studied that the typical pretreatment requires high-energy (steam and electricity) and corrosion-resistant, high-pressure reactors. The fungal pretreatment could potentially lower the severity requirements of acid, temperature and time. These reductions in severity are also expected to result in less biomass degradation and consequently lower inhibitor concentrations compared to conventional thermochemical pretreatment. Furthermore, potential advantages of fungal pretreatment of agricultural residues, such as corn stover, are suggested by its effectiveness in improving the enzymatic cellulose digestibility of corn stover after pretreatment with *Cyathus stercoreus* and *Phanerochaete chrysosporium*.

2.2. AEROBIC COMPOSTING AND ANAEROBIC DIGESTION OF AGRICULTURAL AND HORTICULTURAL WASTES

2.2.1 Total Solids (TS %) and Total Volatile Solids (TVS %)

Singh *et al.* (2012) explained anaerobic digestion as a biological process which involves breakdown of complex substances. The efficiency of digestion system can be evaluated in terms of organics and solid content. Total Solids (TS) and Volatile Solids (VS) were of important considerations in loading the reactor. VS % is the measure of the degradable components that gives the estimates of methane production. It is stated that VS % of cow dung is generally lies between 80 - 86 %.

Agrawal *et al.* (2011) explained that the press mud contains 77 % of volatile solids, lignin, lipids, cellulose, hemicelluloses etc. which favours biogas production. It also has good proportion of nitrogen. This makes it is a very good material for generation of bioenergy (methane) by anaerobic bio methanation.

Budiyono *et al.* (2014) conducted a series of laboratory experiments using 400 ml bio digester operated in batch operation mode. 100 grams of fresh cattle manure (M) was fed to each bio digester and mixed with rumen fluid (R) and tap water (W) in several ratio's (resulted six different M:W:R ratio contents and six different Total Solid (TS) contents. The results showed that the rumen fluid inoculum influenced the biogas production rate and increased more than two times when compared to manure substrate without rumen fluid inoculum.

Ofoefule *et al.* (2010) carried out anaerobic digestion of some animal dung like cow, swine, rabbit, poultry and goat. The TS % in the animal dung was 36.38, 27.01, 15.65, 17.02 and 19.89 % respectively and the TVS % in the animal dung was 77.38, 72.94, 82.35, 83.80 and 68.80 % respectively. The average volume of biogas produced was 44, 40, 37, 33 and 31 dm³ per total mass of slurry respectively. The total volume of biogas produced by each dung for the whole period was compared and cow dung produced highest volume of biogas while goat dung produced least volume.

Rouf *et al.* (2010) worked on the optimization of biogas generation from press mud using batch reactor. Press mud, cow dung and kitchen wastes contain 22.2, 24.9 and 11.4 %, TS and 77.1, 77.2 and 92.0 % TVS respectively.

Yokoyama *et al.* (2007) conducted experiments using cow dung for bio gas production and the composition of cow dung was estimated. Cow dung contains 15 % TS, 85 % TVS and the pH was 6.6.

Osman *et al.* (2006) studied bio gas production from different agricultural wastes like wheat straw, ground nut shells and sugar cane bagasse. TS % in the substrates was 18, 18.2 and 8.85, whereas TVS % was 85, 93.5 and 86.45 respectively.

2.2.2 Total Volatile Fatty Acids (VFA)

Sunil *et al.* (2016) examined the effect of calcium chloride (CaCl_2) on anaerobic digestion of municipal solid waste. Three anaerobic digesters with different concentrations of CaCl_2 , namely sample without additives (Control), sample with 2.5 g l^{-1} CaCl_2 (R_1) and sample with 5 g l^{-1} CaCl_2 (R_2) were studied separately. It was observed that pH decreased with an increase in the dosage of CaCl_2 . Total solids and volatile solids reduction percentage in digester R_2 was considerably lower than Control and R_1 digesters. The significant positive correlation with small increments in volatile solids were observed with an increase in pH. The cumulative biogas production in all the three digesters (Control, R_1 and R_2) were observed to be 35.38, 46.46 and 37.56 l^{-1} respectively. It was also observed that the Volatile Fatty Acids (VFAs) removal efficiency in digester R_1 was the best among all the three digesters.

Kun *et al.* (2014) carried food waste anaerobic fermentation under acidic conditions using inoculum based on aerobic activated sludge or anaerobic activated sludge for volatile fatty acids (VFAs) production. The results showed that food waste hydrolysis increased obviously when inoculum anaerobic activated sludge was used relative to inoculum aerobic activated sludge at any pH investigated. Additionally VFAs production at pH 6.0 was the highest, regardless of the inoculum used. VFAs composition analysis showed that butyrate acid was the prevalent acid at pH 6.0 followed by acetate acid and propionic acid.

Pham *et al.* (2013) recommended methods to measure bio gas production potential of animal manure. VFA in the cow manure and pig manure was 2.67 and 6.65 g kg^{-1} respectively.

Siedlecka *et al.* (2008) revealed that the volatile fatty acids (VFAs) are present in environmental waters in the range of 1 to 5,000 ppm and different methods have been

reported the distillation method followed by potentiometric titration, spectrophotometric and gas chromatographic methods.

Lahav *et al.* (2004) explained that an increase in VFA concentration was the first practical measurable indication that an anaerobic treatment system was in stress. The VFA concentration in properly running anaerobic digesters was typically stable and low and ranging between 0.5-2.0 m mol dm⁻³.

Sans *et al.* (1993) studied the volatile fatty acids (VFA) production by means of anaerobic fermentation of urban organic wastes. The results showed an important VFA production observed at mesophilic temperature (37 °C). Particularly, VFA concentration in the outlet sludge went from 11.8 g l⁻¹ at 2 days of retention time up to 23.1 g l⁻¹ at 6 days of retention time.

2.2.3 pH

Yangyang *et al.* (2016) revealed that the solid-state anaerobic co-digestion of tomato residues with dairy manure and corn stover was conducted at 20 % total solids under 35 °C for 45 days. Results showed digestion of mixed tomato residues with dairy manure and corn stover improved methane yields. The highest VS reduction (46.2 %) and methane yield (415.4 L kg⁻¹ VS feed) were achieved with the ternary mixtures of 33 % corn stover, 54 % dairy manure, and 13 % tomato residues, lead to a 0.5-10.2 fold higher than that of individual feedstocks. Inhibition of Volatile Fatty Acids (VFAs) to biogas production occurred when more than 40 % Tomato residues were added. The results indicated that ternary mixtures diluted the inhibitors that would otherwise cause inhibition in the digestion of tomato residues as a mono-feedstock.

Ukpai *et al.* (2012) investigated anaerobic digestion in generating biogas from three types of wastes: Cow dung, Cowpea and Cassava peeling. During the digestion period, the volume of biogas production and the changes in pH indicated that at neutral pH, the highest peak of gas production was attained and at the slightly acidic pH range, there was no gas production. The cow dung had the highest cumulative biogas yield of 124.3 l⁻¹ per Total Mass of Slurry (TMS) while cow pea had 87.5 l⁻¹ per TMS and Cassava peeling with lowest cumulative biogas yield of 87.1 l⁻¹ per TMS within this retention period. The result obtained from the gas production showed that Cowpea produced the highest methane content of 76.2 %, followed by cow dung with 67.9 % and Cassava peeling has the least methane content of 51.4 %.

Putri *et al.* (2012) indicated that production of biogas from livestock waste manure as one of the alternative utilization of organic wastes. He explained variable A (1:3) ratio and the variable B (1:2) ratio produced the highest volume of biogas compared to other ratios. The highest biogas production occurred on an average at day 23. Generally the addition of water and rumen can increase the production of biogas since both raw materials supporting the two important stage in the biogas production (hydrolysis and acetogenesis) in certain level. The degree of acidity is maintained in the range of 6.6 to 7.6 as methanogenic bacteria can only work at that pH range.

Velmurugan and Alwar (2011) demonstrated that anaerobic digestion of vegetable wastes were anaerobically digested in a fed-batch laboratory scale reactor at mesophilic conditions (35 °C) pH in the range of 6.8 to 7.4 should be maintained in the anaerobic digestion process, which is the optimum range for methanogens growth. The vegetable wastes were fed with an average pH of 5.75 and the reactor residue average pH was 7.55 which was slightly above the optimal pH. The TS % in the substrates were estimated as 6.55 %, 8.50 % and 12.28 % in banana stem, cabbage, ladies finger respectively and the total volatile solids % in the substrates were estimated as 83.21 %, 90.45 % and 90.65 %. Methane yield was 0.3871 CH₄ g⁻¹ VS. The average methane content in the biogas was 65 % and the methane yield was 0.3871 CH₄ g⁻¹ VS added for the selected types of wastes. Hence they concluded that vegetable wastes were potential source for energy production.

Cioabla and Eandionel (2010) mentioned that the quality of the produced biogas is closely related to the type of waste biomass used, but by utilization of the by-products (fertilizer or fuel) the economy of the process might turn into a favourable one. The results showed that the origin and quality of the input raw material used is very important, related with the type of biomass. The duration of the batch fermentation and also the ratio between solid matter and liquid volume. The main parameters influenced for the process of anaerobic fermentation are the temperature regime, the pH of the suspension, the type of biomass and its chemical characteristics.

Espinosa *et al.* (2010) conducted experiment on the short term effects of temperature changes in a pilot plant for the production of biogas from poultry litter. The chemical composition of the substrate, the effluent and the biogas were monitored throughout the experiments. The pH variations in the poultry litter was in between 7.3 and 7.7. Initially pH was 7.5 in the poultry litter and later it was decreased to 7.3 finally the pH was 7.6. The litter was diluted with fresh water to a total concentration of solids

of 5 - 7 %. The average influent chemical oxygen demand was 52 g l⁻¹ while the ammonia concentration was 17 g l⁻¹ throughout the experiment.

Yadvika *et al.* (2004) studied that recirculation of effluent slurry on daily basis and stirring of the digester's contents by using simple techniques for enhancing biogas production seems to be quite viable under rural conditions. Keeping various parameters within the desired range also improves gas production but the practical difficulty lies in maintaining and monitoring these regularly. It is a crucial point which needs due consideration since a slight change in pH or temperature could otherwise result in reduction of gas production. Similarly formation of volatile fatty acids beyond a particular range hinders the methane production. Loading rate and solid concentration should be properly balanced and continuously maintained.

Atiyeh *et al.* (2000) in their research reported that earthworms reduced the pH and decreased the moisture content in the manure. The C: N ratio of the manure with or without earthworms decreased progressively from 36 to 21. The ash and total nitrogen contents increased greatly for a few weeks after the introduction of earthworms, it reflected a rapid breakdown of carbon compounds and mineralization of nitrogen by the earthworms. Earthworms reduced microbial biomass early in the process. But enhanced nitrogen mineralization and increased the rates of conversion of ammonium-nitrogen into nitrate.

2.2.4 Nitrogen (N), phosphorus (P) and Potassium (K) content in different substrates

Wani *et al.* (2013) worked on the nutrient content and different physio-chemical parameters in garden waste, kitchen waste and cow dung. They explained that organic carbon content in the cow dung (18.4 %) is more when compared to the kitchen wastes (13.3 %) and garden wastes (11.7 %). The N (1.97 %) and P (0.62 %) content was more in the cow dung compared to kitchen waste and garden wastes, whereas K content was more in kitchen wastes (0.01 %) when compared to cow dung (0.88 %) and garden wastes (0.60 %).

Hema *et al.* (2012) studied the influence of microbial enrichment on microbial population and nutrient status of organic manures. The studies concluded that the enriched manures, enriched vermicompost had significantly registered more microbial load and high content of N, P, K and micronutrients content followed by enriched compost and bio gas slurry had 1 % N, 0.3 % P and 0.75 % K before enrichment and 1.25 % N, 0.5 % P and 0.88 % K after enrichment.

Garg *et al.* (2011) studied the management of food industry waste by vermicomposting technology. After 15 weeks of vermicomposting significant increase in total nitrogen (60 -214 %), total available phosphorous (35.8–69.6 %), total sodium (39 - 95 %), and total potassium (43.7 - 74.1 %), while decrease in pH (8.45 - 19.7 %), total organic carbon (28.4 - 36.1 %) and C: N ratio (61.2 - 77.8 %) was recorded. The results indicated that food industry waste may be converted into good quality manure by vermicomposting if mixed with other organic wastes in appropriate quantities.

John *et al.* (2011) studied that C/N ratio was reduced from 41.8 to 17.6 and 38.8 to 15.4 in municipal solid waste and cow dung (10:1) respectively. The important nutrients, NPK showed significant ($P < 0.05$) higher content in vermicomposts than worm-unworked composts. The bacterial, fungal and actinomycetes population in vermicompost was significantly increased than in the compost.

Maria *et al.* (2011) studied that impact of the earthworm species *Eisenia fetida* on microbial community structure and function. The activity of earthworms greatly reduced the bacterial and fungal biomass and microbial diversity relative to the control values. However, the pronounced presence of earthworms in the younger layers stimulated microbial activity and as such increased carbon mineralization probably due to the fact that the microorganisms may have been less resource-limited as a result of earthworm activity.

Khan and Ishaq (2011) studied the chemical nutrient analysis of different composts (Vermicompost and Pitcompost) and Garden soil (control) were taken first for chemical analysis and then to find the effect of these composts on the growth of a vegetative growth of '*Pisum sativum*'. It was found that the vermicompost was rich in nutrients like potassium, nitrate, sodium, calcium, magnesium, and chloride and have the potential for improving plant growth than pit compost and garden soil (control). The optimal plant growth was found in pots containing vermicompost. The study also showed distinct differences between vermicompost, pit compost and garden soil (control) in terms of their nutrient content and their effect on plant growth.

Theunissen *et al.* (2010) reported that potential of vermicompost produced from plant waste on the growth and nutrient status in vegetable production. Vermicompost contains plant nutrients including N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu and B, the uptake of which has a positive effect on plant nutrition, photosynthesis, the chlorophyll content of the leaves and improves the nutrient content of the different plant components (roots,

shoots and the fruits). The high percentage of humic acids in vermicompost contributes to plant health, as it promotes the synthesis of phenolic compounds such as anthocyanins and flavonoids which improves the plant quality and act as a deterrent to pests and diseases.

Swati and Reddy (2010) reported on nutrient status of vermicompost of vegetable market waste and floral waste processed by three species of earthworms namely, *Eudrilus eugeniae*, *Eisenia fetida* and *Perionyx excavates*. The nutrients nitrogen, phosphorus, potassium, calcium and magnesium increased in the vermicompost and compost while the organic carbon, C : N and C : P ratios decreased as the composting process progressed from 0 to 15, 30, 45 and 60 days. The nutrient status of vermicompost from all earthworm species produced from both the wastes were more than that of the compost and that of their respective substrates.

Islam *et al.* (2010) examined the effectiveness of biogas slurry (liquor from anaerobic digestion process) as a nitrogen source for the production of maize fodder (*Zea mays*). They reported that biogas slurry had 5.88 mg kg⁻¹ N, 2.72 mg kg⁻¹ P, 1.33 mg kg⁻¹ K and 0.79 mg kg⁻¹ S.

Kolar *et al.* (2010) tested the procedure of combined phytomass utilization Integrated Generation of Solid Fuel and Biogas from Biomass (IFBB) proposed for ensiled grass matter from the aspect of suitability of its use for a typical substrates as a mixture of cattle slurry, maize silage and grass haylage. N, P and K analysis of the substrates was carried and concluded that cattle slurry was with more nutrients 2.4, 1.3 and 5.3 % compared to maize silage 0.1, 0.2 and 1.4 % respectively.

Gorakhnath *et al.* (2009) evaluated that potential of an epigeic earthworm *Eisenia fetida* to convert the different combination of variety of wastes in to rich nutrient vermicomposts or vermiwash and pre and post chemical analysis of feed mixtures. Vermicomposting resulted in significant decreases in pH, total organic carbon, electrical conductivity and C : N ratio while significant increase in total nitrogen available phosphorus, exchangeable potassium and calcium in vermicompost or vermiwash. The increased level of plant nutrients in final products in different organic resources demonstrated that the vermicompost or vermiwash of these wastes will be a valuable as a bio fertilizer for sustainable land restoration practices.

Meenatchi *et al.* (2009) conducted experiment on the microbial load of vermicompost and vermiwash prepared by using different food substrates and earthworm

species. The results revealed that the best substrate- earthworm combination beneficial microflora such as highest bacterial population obtained from kitchen waste + *Perionyx excavatus* (65×10^7 CFU/g⁻¹), check + *Perionyx excavates* (10×10^3 CFU/g⁻¹) recorded highest fungal population and check + *Perionyx excavatus* (15×10^4 CFU/g⁻¹) recorded maximum actinomycetes population. Vermiwash obtained from Paddy straw + *Perionyx excavatus* recorded maximum bacterial population (67×10^7 CFU/ml⁻¹) and check + *Perionyx excavatus* recorded maximum fungal population (10×10^3 CFU/ml⁻¹). Vermiwash obtained from soybean harvest waste + *Eudrilus eugeniae* recorded maximum actinomycetes population (14×10^3 CFU/ml⁻¹).

Birnin *et al.* (2009) worked on the anaerobic fermentation of cattle dung and carried out to determine their biogas yield potentials. The N, P and K analysis on the samples showed more contents in undigested sample of A and contrarily, higher contents in digested sample of cattle dung. The N, P and K in cattle dung undigested samples was 1.12, 0.25 and 10.28 %. Whereas the N, P and K in cattle dung egret droppings digested samples was 1.72, 0.61 and 10.28 % respectively.

Muthukumarvel *et al.* (2008) studied that vermicomposting of vegetable waste used cow dung to find out the possibility of utilization of vegetable waste for vermiculture. Earthworm *Megascolex mauritii* cultured in plastic trays (45 x 30 x 30 cm) containing soil alone (control) (T₁), soil + cow dung (T₂), soil + vegetable waste (T₃) and soil + vegetable waste + cow dung (T₄) for 60 days. Nutrient values were determined from the compost and compared with that of the control. It was found that NPK values were maximum in compost obtained from vegetable waste with the use of cow dung.

Chhotu *et al.* (2008) studied that vermicomposting of vegetable waste: a biophysicochemical process based on Hydro-Based Operating Bioreactor (HBOB). The vermicompost developed in the Hydro-Based Operating Bioreactor was found to have comparatively high value of nutrients such as calcium, sodium, magnesium, iron, zinc, manganese and copper which can serve as a natural fertilizer giving higher yields of plants. The vermicomposting proved very effective and efficient for developing compost from vegetable waste.

Bernal *et al.* (1998) established the maturity indices as $C : N < 12$, $NH_4 : NO_3 < 0.16$ and $NH_4-N < 0.04$. In addition, compost maturity was also determined by carbon mineralization. During sewage sludge composting, when rice husk was added, loss of N was reduced.

2.2.5 Organic Carbon (OC)

Priyanka *et al.* (2015) explained that the residual effect of inorganic and organic fertilizer input on selective soil parameter such as pH and available nitrogen which are crucial in terms of earthworm survival and abundance. Residual effects of applied organic and inorganic soil fertilizers on soil properties were found to vary based on different factors including type, rate and timing of fertilizer application and soil characteristics. Results of their investigation revealed that changes in soil available nitrogen and soil pH leaving acidic effect on the vermicompost which predominantly affected earthworm survival.

Yadav *et al.* (2013) conducted experiments and showed results that indicated cow dung and bio gas plant slurry can be used as a raw material in vermicomposting. The substrate cow dung has an E.C of 1.2 to 2.2 dsm^{-1} and biogas plant slurry with an E.C of 1.0 to 1.4 dsm^{-1} . The nutrients in the cow dung like N, P and K were 6.5 to 8.6 g kg^{-1} , 5.0 to 8.7 g kg^{-1} and 2.8 to 7.8 g kg^{-1} respectively. While in the biogas plant slurry the N, P and K were 5.2 to 10 g kg^{-1} , 5.0 to 5.8 g kg^{-1} and 1.3 to 4.2 g kg^{-1} respectively. The organic carbon content was 425 to 550 g kg^{-1} for cow dung and 400 to 470 g kg^{-1} for biogas plant slurry.

Stumpe *et al.* (2012) studied that organic carbon dynamics and enzyme activities in agricultural soils amended with biogas slurry, liquid manure and sewage sludge. The organic carbon % in the biogas slurry was 23.2 %.

Narkhede *et al.* (2010) examined the variability which occurs in key parameters like pH, temperature, moisture content and organic carbon during the 41 days regular monitoring of composting process. A 25 kg of municipal solid waste was mixed with 5 kg of sewage sludge so as to make 5:1 ratio for composting. pH ranging 7.1 in the first phase, 5.9 in the middle phase, 8.5 at the end stage and then become stable. Temperature rise from the first day of process and become 60 °C on 26 day then decreased to 28 °C at the end of process and became practically constant after 35 days. Moisture content in compost was unstable throughout the process due to changing microbial population. Organic carbon was 30 % and gradually increased to 59 % in distinct time of interval. The results obtained from composting treatment given to MSW and sewage sludge indicate that combined composting will be an attractive method for management of municipal solid waste and disposal of sewage sludge in the Indian context.

Suthar (2010) worked on the recycling of agro industrial sludge through vermitechnology. Vermicomposting bedding material was analysed for its different physico chemical parameters. The pH of the cow dung was 8.5 and in bio gas slurry the pH was 7.3. The organic carbon in the cow dung was 329.8 g kg^{-1} , in biogas slurry it was 359.5 g kg^{-1} and in vegetable wastes was 390.1 g kg^{-1} .

Bernal *et al.* (2009) worked on the composting of animal manures. The pH in the animal manures was 7.9 - 8.8 and in solid poultry manure was 7.6. The organic carbon content in liquid poultry manure was $11\text{-}112 \text{ g kg}^{-1}$ and in solid poultry manure was $103\text{-}597 \text{ g kg}^{-1}$.

Bouallagui *et al.* (2004) explained the psychrophilic, mesophilic and thermophilic process that has been used in the laboratory scale experiments of fruit and vegetable wastes for anaerobic digestion. The TS % in the fruit and vegetable wastes substrates was found to be 11 % on an average. The organic carbon content of the substrate was found to be 50.9 % on an average.

2.2.6 Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

Sangeetha and Sivakumar (2016) clearly explained that the anaerobic digestion was employed for reduction of chemical oxygen demand (COD) and maximization of biogas production using synthetic sago wastewater by batch process. The optimum condition in which maximum COD removal (81.85 %), biological oxygen demand (BOD) removal (91.61 %) and biogas production of 99.4 ml day^{-1} were achieved at pH 7.0 with an initial BOD of 1374 mg l^{-1} and with the retention time of 10 days at 32°C . Maximum COD reduction and biogas production were achieved at a pH 7.0 initial BOD of 1374 mg l^{-1} , temperature of 32°C and with the retention time of 10 days.

Kavya *et al.* (2015) explained that cow dung along with other agricultural wastes (press mud, poultry litter, kitchen wastes, maize stalk and fruit wastes) were used for the biogas production in lab scale. For each treatment 750 gr of substrate and 1500 ml of water was used in inoculum mixture in 3 liters glass bottles. Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) was estimated at various stages of gas production. BOD and COD of the slurry samples was more in the substrates initially before anaerobic digestion but later reduced gradually by the end of the gas production which implies the effect of anaerobic digestion on the reduction of BOD and COD and this indirectly leads to the reduction in the environmental pollution.

Owczuk *et al.* (2013) explained that waste products from agricultural industry is a valuable source of feedstock for biogas production. Seven different kinds of feedstocks were loaded into the digesters (different swine manure to Maize silage ratios). It was found that the cumulative biogas production increased with increasing proportions of the maize silage. However, the methane production was achieved more slowly at higher rather than lower organic loadings. The volatile solids % in the swine manure and maize silage was 60.9 % and 95.9 % respectively. The pH of swine manure and Maize silage used was 7.25 and 6.88 respectively. During the anaerobic digestion the pH variation was in between 7.05 and 7.65. The COD of the Maize silage was 285 mg O₂ per gram.

Salunkhe *et al.* (2012) clearly explained about kitchen solid waste i.e. food waste based biogas system was more useful than conventional i.e., cow dung based biogas system. Slurry generated in kitchen solid waste based biogas digester contains less BOD as compared to conventional biogas plant. BOD of the kitchen solid waste slurry was 18 mg l⁻¹ whereas for cow dung slurry was 30 mg l⁻¹. Nutrients NPK in wet slurry of kitchen waste was 1.96, 0.96 and 3.32 % respectively and NPK in dry slurry were 0.98, 0.84 and 1.37 % respectively. But NPK in cow dung slurry was 0.56, 0.35 and 0.78 % respectively. NPK in slurry were four times more than that of cow dung.

Stephanie *et al.* (2008) suggested that the effluent water has significant lower Chemical Oxygen Demand (COD) and higher dissolved nutrient concentration than the influent water which increases the usefulness of the effluent as an organic fertilizer and decreases its organic loading on surface waters. The results suggest that the systems have the ability to withstand fluctuations in the influent water quality. Their study revealed that small-scale agricultural digesters can produce methane at concentrations useful for cooking, while improving the quality of the livestock wastewater.

2.3. METHANE PERCENTAGE AND ALCOHOL CONTENT:

2.3.1 Bio Gas Production and Methane Percentage

Aragaw *et al.* (2013) suggested that anaerobic co-digestion strategies are needed to enhance biogas production when treating certain residues such as cattle/pig manure. Co-digestion of food waste with animal manure or other feedstock's with low carbon content can improve process stability and methane production. In this study, anaerobic digestion and co-digestion of cattle manure with organic kitchen waste using rumen fluid as inoculum have been experimentally tested to determine the biogas potential. Co-

digestion substantially increased the biogas yields by 24 to 47 % over the control (organic kitchen waste and dairy manure only). The highest methane yield of 14,653.5 ml was obtained with 75 % Organic Kitchen Waste (OKW) and 25 % cattle manure additions. In contrast, addition of 75 % cattle manure caused inhibition of the anaerobic digestion process and its cumulative methane yield was 23 % lower than that with 25 % cattle manure addition.

Chamarthi *et al.* (2013) explained that the temperature plays a crucial role in fermentation of the slurry for the better formation of methanogens. An increased temperature in the digester tank facilitates faster reaction rates and hence faster gas yields are produced. The gas samples which are collected through gas balloons from the biogas plant are taken to the laboratory for determination of composition of biogas by using gas chromatography. The results reviewed that methane composition is increased from 63.40 % to 64.22 %. The greater methane yields result in better performance of the plant and proves to be more effective when compared to that of mild steel dome digester.

Prabhudessai *et al.* (2013) worked on anaerobic digestion of locally available agro wastes like coconut oil cake, cashew apple waste and grass from lawn cuttings. The most productive agro waste, in terms of methane yield was coconut oil cake and grass. The results showed that the initial volatile solids concentration significantly affected the biogas production. The methane yield from coconut oil cake was found to be 383 ml and 277 ml added at 4 and 4.5 ml g⁻¹ VS per litre. In case of grass the biogas production increased with increasing VS concentrations with methane yield of 199, 250, 256, 284, and 332 ml at 3, 3.5, 4, 4.5, and 5.0 ml/ g VS per litre. For cashew apple waste single-stage fermentation inhibited biogas production. However, phase separation showed methane yield of 60.7 ml and 64.6 ml at 3.5 and 4.0 g VS per litre, respectively. The anaerobic biodegradability of coconut oil cake was evaluated in fed batch mode in a 5 l⁻¹ anaerobic reactor at 4 g VS per litre per batch and the maximum methane yield was found to be 320 ml.

Dubrovskis *et al.* (2012) studied the potential of biogas production from various raw materials, using available and cheaper raw materials. Biomass mixed within inoculums (fermented cow manure) was investigated for biogas production. Further increase of biogas and methane output from leaves or straw biomass can be provided by thermal and/or chemical pre-treatment of biomass to improve biodegradability of lignin. The average methane yield from chopped barley straw was 280 ± 39 l⁻¹ kg VSA-1 and

average methane content was 64 %. Addition of carbon rich biomass is recommended for anaerobic fermentation will increase the substrates C : N ratio.

Eze and Ojike (2012) concluded that the anaerobic digestion through maize chaffs, stalks and cobs have the potential to generate biogas and the chaffs produced more biogas than the other two wastes. It was also observed that the chaffs generated more methane gas than the other two and was the earliest to flame when tested. Maize chaffs, stalks and cobs were shredded and mixed in water to waste ratios ranging from 3:1 to 4:1 (water to wastes) and anaerobically digested in three separate 0.1 m³ digesters for 30 days.

Narayani *et al.* (2012) explained that the cumulative biogas production increased, when fruit wastes along with co substrates were used. Highest biogas production (405 ml) was obtained for 75 % fruit waste + 25 % cow dung sample, whereas lowest for the sample with 75 % fruit waste + 25 % rice bran. Also the methane % was highest when the fruit waste digested with cow dung (80 %) compared to the other samples. Anaerobic digestion with cow dung has greater potential for bio gas production because the presence of anaerobes that lead to enhancement of biogas production. Rice bran and fruit waste alone does not have potential for biogas production.

Rajput *et al.* (2012) found that waste generated in the biogas power plant is harmful not as economic point of view but it is highly polluted and hazardous for human beings. However this waste can be further anaerobically digested and that will be not only eco-friendly but also it will generate biogas as high quality manure. For rapid digestion it has been found that slurry with PW in 60:40 leads to high gas yield. Methane content in the generated biogas is also more than 47 % which is sufficient to run gas engines in power plant. Maximum gas generation was recorded in 60th day i.e., about (0.45 l g⁻¹ VS.).

Ilaboya *et al.* (2010) conducted a survey to ascertain the amount of biogas that can be generated from various feed stock. A practical laboratory scale experimental design using agricultural waste was also done to find out the effects of alkaline [NaOH] on the volume of biogas generated using a mixture of Pineapple, Plantain and Cassava peelings as the feed stock. Results revealed that a high volume of gas generated when the operating conditions inside the digester is maintained at moderately alkaline condition. Further findings also revealed that the digester temperature remained within the range of 27 to 35.5 °C throughout the period of experimentation.

Fantozzi and Buretti (2009) designed a single-stage, batch, mixed laboratory scale mesophilic anaerobic digester and a good productivity for pig manure was found to be

0.13 Nm³ kg⁻¹ VS and a high reduction of nitrogen content (poultry litter: 86 %). Pig manure anaerobically digested when compared with chicken manure the highest methane production was found to be 0.11 Nm³ kg⁻¹ VS. When compared pig manure to rumen fluid on olive husk, the first one showed better performance (0.11 vs. 0.03 Nm³ kg⁻¹ VS).

Sagagi *et al.* (2009) explained results of the study on biogas production from fruits and vegetables waste materials and their effect on plants when used as fertilizer (Using digested and undigested sludge). It has been observed that the highest weekly individual production rate was recorded for the cow dung (control) slurry with average production of 1554 cm³, followed by pineapple waste which had 965 cm³ of biogas, then orange waste which had 612 cm³ of biogas, lastly, pumpkin and spinach wastes had 373 cm³ and 269 cm³ respectively. The results obtained showed that difference in the production of biogas to a large extent depends on the nature of the substrate. All the substrates used appeared to be good materials for biogas production and their spent slurries can be used as a source of plant nutrients.

Tiquia *et al.* (1998) concluded that high moisture content may produce anaerobic conditions, which will prevent and halt the ongoing composting activities.

2.3.2 Alcohol percentage

Snehal *et al.* (2014) focused his study on exploitation of banana pseudo stem as a source for bioethanol production from the sugars released due to different chemical and biological pre-treatments. Two fungal strains *Aspergillus ellipticus* and *Aspergillus fumigatus* reported to be producing cellulolytic enzymes on sugarcane bagasse were used under co-culture fermentation on banana pseudo stem to degrade holo cellulose and facilitate maximum release of reducing sugars. Fermentation of cellulosic hydrolysate (4.1 g %) gave maximum ethanol (17.1 g l⁻¹). It was observed that pre-treated banana pseudo stem can be economically utilized as a cheaper substrate for ethanol production.

Sandesh *et al.* (2014) concluded that the production of bioethanol as a fossil fuel additive to decrease environmental pollution and reduce the stress of the decline in crude oil availability is becoming popular. Its production mainly utilizes three types of raw materials sugar juice, starchy crops and lignocellulosic materials. This research work investigated ethanol production from fruit juices of four different fruits such as *Vitis vinifera* (Grapes), (*Saccharum officinarum*) (Sugarcane), *Citrus cimetta* (Mosambi) and *Citrullus canatus* (Watermelon) using *Saccharomyces cerevisiae* for fermentation and

optimizing several factors that influence the process for bioethanol production such as temperature, pH and sugar concentration.

Girish *et al.* (2014) studied that bio ethanol mainly produced from biological methods involving fermentation of cellulosic biomass in a broad spectrum. Hence, bioethanol production from three different decaying citrus fruits like *Citrus sinensis*, *Citrus limetta* and *Ananas comosus* were studied. The sugar content before and after fermentation was analyzed by DNS method, the result showed that the sugar content was more before fermentation (*Citrus limetta* 21 mg ml⁻¹, *Ananas comosus* 20 mg ml⁻¹ and *Citrus sinensis* 17 mg ml⁻¹) when compared to after fermentation (*Citrus limetta* 16 mg ml⁻¹, *Citrus sinensis* 12.5 mg ml⁻¹ and *Ananas comosus* 11 mg ml⁻¹). The production of ethanol was higher in *Ananas comosus* (13 %) than the other two *Citrus limetta* (12 %), *Citrus sinensis* (10 %) which was calculated by distillation method.

Itelima *et al.* (2013) studied that the waste from fruits such as banana, plantain and pineapple peels are used for the production showed that after 7 days of fermentation, pineapple peels had the highest biomass yield of 1.89 (OD), followed by banana peels 1.60 (OD), while plantain peels had the least 0.98 (OD). The reducing sugar concentrations ranged between 0.27 – 0.94 mg cm³ for pineapple, 0.20 – 0.82 mg cm³ for banana and 0.16 – 0.45 mg cm³. The optimal ethanol yields were 8.34 % v/v, 7.45 % v/v and 3.98 % v/v for Pineapple, Banana and Plantain peels respectively. The results indicated that pineapple and banana peels ethanol yields were significantly higher ($P < 0.05$) than plantain peel ethanol yield.

Jayant *et al.* (2012) studied that the ethanol produced from fruits of pineapple, orange and sweet lime. Effect of different constant times 24 and 72 h, and 8 days (in submerged fermentation) pH (3.5 to 8.5), temperature (78 °C) and autoclaved pre-treatment (121 °C, 20 min) was also studied to improve the yield of ethanol in fruits. Yeast (*Saccharomyces cerevisiae* and *Candida albicans*) are used for fermentation. The results showed that there is a substantial increase in the quantity of ethanol produced in submerged fermentation as compared to that produced by the solid state fermentation. Optimal pH and temperatures for the better yield of ethanol were 3.5 to 8.5 and 78 °C respectively.

Nibedita *et al.* (2012) explained that the conventional crops such as corn and sugarcane are unable to meet the global demand of bioethanol production due to their primary value of food and feed. Therefore, lignocellulosic substances such as agricultural

waste are attractive feed stocks for bioethanol production. Bioethanol from agricultural waste could be a promising technology though the process has several challenges and limitations such as biomass transport, handling and efficient pre-treatment methods for total delignification of lignocelluloses. Proper pre-treatment methods can increase concentrations of fermentable sugars after enzymatic saccharification, thereby improving the efficiency of the whole process.

Rabelo *et al.* (2011) studied the potential of biogas production from the residues of bagasse was pre-treated, enzymatically hydrolysed and the waste from pre-treatment and hydrolysis were used to produce biogas. Evaluation of four different biofuel production scenarios has shown that 63 - 65 % of the energy that would be produced by bagasse incineration can be recovered by combining ethanol production with the combustion of lignin and hydrolysis residues, along with the anaerobic digestion of pre-treatment liquors, while only 32 - 33 % of the energy is recovered by bioethanol production alone.

Balasubramanian *et al.* (2011) found that India is the second major producer of fruits and vegetables. In the world, particularly grape orange, apple, pineapple, banana and papaya. Some micro-organisms were isolated from different fruits, organisms like *Acetobacter aceti*, *Clostridium butyrium*, *Bacillus* sp and *Micrococcus*, some fungal microbes like *Aspergillus flavus*, *A. niger*, *penicillium chrysogenum* and *Fusarium* species were best for fermentation process and gives the nutritive values. The maximum amount of nutritive compounds were present in papaya; i.e., calcium 8.5 to 93.1 %, phosphorus 6.8 to 8.9 %, protein 0.47 to 2.15 and fat 0.04 to 0.5 % respectively. The papaya fruit pulp from raw starch hydrolase are attractive resources for economical production of ethanol.

Borah and Mishra (2011) concluded that demand of petroleum products and the prices of petrol & diesel are increasing world-wide. Fruit wastes (apple pomace and rotten banana) can be used as a substrate for the production of ethanol by treating it with distilled water, small amount of sucrose and *Saccharomyces cerevisiae* was added and produced ethanol. After distillation they could recover a total volume of 200 ml of 48 % concentrated ethanol from a total volume of 1500 ml of substrate mixture. By redistilling they obtained higher concentration of ethanol.

Sudarshan *et al.* (2011) explained that the fermentation process from sugarcane can be carried out by the methods. One by using simple sealed glass container and second

by using a fermenter. In this context a comparative study was carried out between lab scale fermenter and sterilized glass vessel for production of ethanol from fruits of *Vitislana roxburgh* which are rich in fermentable sugars. *Saccharomyces cerevisiae* NCIM-3176 was used to initiate fermentation in fermenter and glass vessel respectively. Production of ethanol increased by 1 % in fermenter (10.1 % v/v) as compared to glass vessel (9.05 % v/v). Similarly total acidity was found to be high in fermenter (0.78 %) than glass container (0.62 %). The present investigation indicated that fermenter is more efficient system for ethanol production from fruits of *Vitislana roxburgh*.

Hossain *et al.* (2011) found that the banana waste was selected for the production of bioethanol by using *Saccharomyces cerevisiae*. The effects of various operating conditions were recorded which includes different temperatures, shaking period and saccharification. Overall, the fermented banana fruit waste produced 4.1 to 7.1 % bioethanol. The bioethanol yield from mixture of rotten banana fruit increased with increase fermentation period. It also increased with yeast concentration, using 35 % of water at 35 °C. Fermented banana treated with mixture of enzymes was the best method used for higher bioethanol production.

Manoj *et al.* (2011) concluded that ethanologenic yeasts were isolated from different sources like spoiled fruits, market yard wastes, industrial wastes and orchard soils. Cultures were identified and screened for alcohol production, AMY-2 was found to be the best among the isolates. Over ripened sapota fruit pulp with reducing sugars 7.62 (g 100 g⁻¹), total sugars 11.85 (100 g⁻¹), total soluble solids 19.50 (°Brix) and pH 5.4 was used for fermentative production of alcohol. About 30.17- 67.00 (g l⁻¹) of alcohol was obtained when fermentation was conducted at different pH's (4.0, 4.5 & 5.0), temperatures (ambient temp- 30-38.6 °C & controlled temp- 26 °C) and with cultures (standard culture *Saccharomyces cerevisiae*- MTCC-172 & local best isolate AMY-2) for 72 h. It is established that over ripened sapota fruits can be used effectively for fermentative production of alcohol at pH 5.0 and temperature 26 °C as fermentation conditions in 24 h.

Mishra *et al.* (2007) concluded that total soluble proteins and total phenols content has been found to be increased during submerged fermentation of rice straw by *cellulomonas* and *P. chrysosporium* strains.

Chapter III

MATERIAL AND METHODS

Material and the methods adopted during the course of investigation are presented in this chapter. Analysis was conducted in the Department of Agricultural Microbiology and Bioenergy, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad.

The general laboratory techniques followed in the present study was described by Cappuccino and Sherman (1992) and Aneja (2001) for preparation of media, sterilization, isolation and enumeration of microorganisms, with slight modifications wherever necessary.

3.1 Glass ware

Petri plates, test tubes, microscopic slides, conical flasks of different capacities *i.e.*, 1000, 500 and 250 ml, pipettes of 1, 2.2, 5 and 10 ml, beakers and measuring cylinders of 50, 100, 500, 1000 and 2000 ml were used.

3.1.1 Cleaning of glass ware

Glass ware was first washed with a detergent, then cleaned with tap water and finally placed in the chromic acid solution prepared with following composition:

Chemical	
Potassium dichromate	60 g
Conc. H ₂ SO ₄	60 ml
Distilled water	1000 ml

The glass ware kept in the cleaning solution for 24 h and then thoroughly washed with running tap water before its final cleaning with distilled water and oven dried.

3.1.2 Sterilization of glassware and media

Glassware like pipettes were sterilized in the hot air oven at 160 °C for 1 – 1/2 h before use. Petri plates, test tubes with saline and media were used in the experiment to

estimate the microbial counts. The media like Cellulose Congo Red Agar medium, Nutrient Agar medium etc. and distilled water were sterilized in an autoclave at 15 lb psi (121 °C) for 20 min.

3.2 EQUIPMENT AND APPARATUS USED

Samples were weighed using a single pan electric balance. Hot air oven and autoclaves were used for sterilization of heat stable materials like pipettes and media respectively. Cyclomixer was used for homogenization of sample during serial dilution preparation. Laminar air flow chamber and plate mixer or master spreader were used for spread plate technique. Orbital shaker incubator was used for incubation of enzyme production medium at 120 rpm for 3 days. Quebech colony counter was used for counting the viable population of microorganisms. Centrifuge were used for collection of culture filtrate for cellulose enzyme estimation. Reducing sugars were estimated spectrophotometrically or colorimetrically with 3, 5 – dinitrosalicylic acid using glucose as standards. BOD incubators were used for incubating the samples in BOD bottles to estimate the BOD in the slurry samples. COD apparatus was used for estimating COD in the slurry samples. Elico CM 180 pH meter was used to estimate the pH in the different samples. Mechanical shaker for shaking the broth tubes during the experimental procedures. Kjeldhal apparatus was used to estimate N in the slurry samples. Spectrophotometer was used to estimate the P content in the samples. Flame photometer was used to estimate potassium content in the slurry samples. Muffle furnace was used to estimate the total volatile solids in the slurry samples. Gas chromatography was used for methane (bio gas) estimation

3.3 CHEMICALS USED

The chemicals used in the present investigation were of analytical grade. The pH of the media was adjusted to the required level using 1N NaOH and 1N HCl. Formaldehyde 10 % solution was used to fumigate the incubator and BOD incubator for disinfection.

3.4 PREPARATION OF MEDIA

3.4.1 Cellulose congo red Agar medium

Medium was prepared by taking 1.88 g Carboxymethyle cellulose, 0.5 g Sodium citrate, 7.0 g K₂HPO₄, and 2.0 g KH₂PO₄, 1.0 g (NH₄)₂ SO₄, 0.1 g MgSO₄.7H₂O, 10 g

Agar, and 0.20 g Congo red and 1000 ml Distilled water, at pH 7.0 (Koteswararao *et al.* 2014)

3.4.2 Nutrient Agar medium

Medium was prepared by taking 5.0 g peptone, 3.0 g beef extract/yeast extract, 15 g agar, 5.0 g NaCl in 1000 ml distilled water and pH adjusted to neutral (6.8) at 25 °C.

3.5 COLLECTION OF DIFFERENT SAMPLES FOR ISOLATION OF CELLULASE DEGRADING BACTERIA

3.5.1 Soil sample collection

Different soil samples were collected from surrounding areas in the college of agriculture, PJTSAU, Rajendranagar. One collected from agricultural college, student farm soil, one sample collected from farmer's fields, one sample collected from forest soil and another sample was collected from horse dung dump sample nearby college. These soil samples were air dried and mixed thoroughly and sieved through a 2 mm sieve constituted the soil sample.

3.5.2 Waste collection

Similarly samples were collected from waste receiving dustbin of Domestic kitchen wastes sample, Municipal solid waste sample, Sewage water sample and cow dung sample were collected in pre-sterilized screw cap glass vials of 30 ml capacity were used. Randomly selected one sq.cm blokes of waste patch were scrapped with spatula and collected in separate vials and these were brought to laboratory for isolation of cellulose degrading bacteria. The soil samples were collected from different location after collection, the samples were stored at 4 °C in sterile containers.

3.5.3 Isolation of Cellulose degrading Bacteria

For each soil sample, several sub-samples were taken, homogenized in sterile distilled water containing 0.85 % NaCl (w/v) and serially diluted and pour plate and spread plate techniques were done using Carboxy Methyl Cellulose (CMC) agar media contains 1.0 g peptone, 1.0 g Carboxy Methyl Cellulose (CMC), 0.2 g K₂HPO₄, 1 g Agar, 0.03 g MgSO₄·7H₂O, 0.25 g (NH₄)₂ SO₄ and 0.2 g gelatin and 1000 ml distilled water at pH 7.0 for 48 h of incubation at 30 °C. The plates were incubated at 37 °C for 24-48 h. The formation of a clear zone of hydrolysis indicated cellulose degradation. The ratio of the clear zone diameter to colony diameter was measured in order to select for the highest

cellulase activity producer. Qualitatively the largest ratio was assumed to contain the highest activity.

3.5.4 Identification of the Isolates

The bacterial isolates were presumptively identified by means of morphological examination and biochemical characterisation. The parameters investigated included colony characteristics, shape, size, spore, motility, Gram's reaction, catalase production, urease production, IMVIC tests like Indole test, Methyl red (MR) reaction, Voges-Proskauer (VP) reaction, Citrate utilization, Nitrate reduction, Carbohydrate metabolism (acid-gas production), Starch hydrolysis, Gelatin hydrolysis, Casein hydrolysis, Growth at different pH and Temperatures, Pectin hydrolysis and chitin hydrolysis test and sugar/carbohydrate utilization tests were carried out by following standard methods described in Bergey's Manual of Systematic Bacteriology (Holt *et al.*, 1984).

3.5.4.1 Morphological Characterization

All the isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by Bartholomew and Mittewar (1950).

3.5.4.2 Colony Morphology

Morphological characteristics of the colony of each isolate were examined on Nutrient agar and specialized medium and incubated for according to isolate. Cultural characterization of isolates observed by different characteristics of colonies such as shape, size, elevation, surface, margin, colour, odour, pigmentation, etc were recorded.

3.5.4.3 Gram's Staining

A drop of sterile distilled water was placed in the center of glass slide. A loopful of inoculum from young culture was taken, mixed with water, and placed in the center of the slide. The suspension was spread out on slide using the tip of inoculation needle to make a thin suspension. The smear was dried in air and fixed through mild heating by passing the slide 3 to 4 times over the flame. The smear was then flooded with crystal violet solution for 1 min and washed gently with flow of tap water. Then the slide was flooded with iodine solution. After incubation at room temperature for 1 min, iodine solution was drained out followed by washing with 95 % ethanol. After that, it was washed with water within 15 to 30 sec and blot carefully. The smear was incubated with

safranin solution for 1 min. The slide was washed gently in flow of tap water and dried in air. The slide was examined under microscope at 100 X power with oil immersion and data were recorded.

3.5.5 Biochemical and Physiological Characterization

Different biochemical tests performed and the protocols followed are briefly outlined below.

3.5.5.1 Starch Hydrolysis

Sterile starch agar plates were spotted with 10 µl overnight broth cultures of the isolates and incubated at 28 ± 2 °C for 24-48 h. After incubation, the plates were flooded with iodine solution. The formation of a transparent zone around the colony was taken as positive reaction for the test.

3.5.5.2 Hydrogen Sulfide Test

Sterilized Hydrogen sulphide - Indole - Motility agar (SIM agar) stabs were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48 h at 28 ± 2 °C. Visualization of black colour along the line of inoculation indicated a positive reaction for the test.

3.5.5.3 Indole Production

Sterilized SIM agar slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28 ± 2 °C. Following incubation, 10 drops of Kovac's indole reagent were added to each tube. The isolates showing production of red colour were recorded as positive for indole production.

3.5.5.4 Catalase Test

This test was performed to study the presence of catalase enzyme in bacterial colonies. Fresh cultures of pure isolates were taken on glass slides and one drop of H₂O₂ (30 %) was added. Appearance of gas bubble indicated the presence of catalase enzyme.

3.5.5.5 Oxidase Test

The overnight cultures of the test isolates were spotted on plates poured with sterile trypticase soy agar (TSA) and the plates were incubated for 24 h at 28 ± 2 °C. After incubation, 2-3 drops of N, N, N', N'- tetramethyl- p-phenylenediamine dihydrochloride

(Wurster's reagent) were added onto the surface of growth of each test organism. The isolates showing change of colour to maroon were noted as oxidase positive.

3.5.5.6 Gelatin liquefaction

The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated for 24 h at 28 ± 2 °C. Then the tubes were kept in the refrigerator for 30 minutes at 4 °C. The isolates showing liquefied gelatin were taken as positive and those which resulted in solidification of gelatin on refrigeration were recorded as negative for the test.

3.5.5.7 Methyl Red Test

Sterilized glucose- phosphate broth tubes were inoculated with the test culture and incubated at 28 ± 2 °C for 48 h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Red colour production was taken as positive and yellow colour production was taken as negative for the test.

3.5.5.8 Voges Prausker's Test

To the pre-sterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37 °C for 48 h. After incubation ten drops of Baritt's reagent A was added and gently shaken followed by addition of 10 drops of Baritt's reagent B. Development of pink colour in the broth was taken as positive for the test.

3.5.5.9 Citrate Utilization

Isolates were streaked on Simmon's citrate agar slants and incubated at 28 ± 2 °C for 24 h. Change in colour from green to blue indicates the positive reaction for citrate utilization.

3.5.5.10 Denitrification test

Sterilized nitrate broth tubes inserted with Durham's tube in inverted position were inoculated with overnight grown cultures of the test organisms and incubated at 25 °C for 10-15 days. After incubation, the isolates which showed accumulation of gas in the Durham's tubes were scored as positive for denitrification.

3.5.5.11 Carbohydrate Utilization

All pure bacterial isolates were screened for the carbohydrate fermentation abilities using 4 different carbohydrates (lactose, sucrose, dextrose and mannitol) in peptone broth medium. Bacterial isolates were inoculated in broth containing specific carbohydrate. The change in colour of Peptone broth was observed for utilization of particular carbohydrate present in broth.

3.5.6 Quantitative determination of Cellulase Activity of bacterial isolates

Cellulase activity was assayed by using dinitrosalicylic acid (DNS) reagent by estimation of reducing sugars released from CMC solubilized in 0.05 M phosphate buffer at pH 8.0. The culture broth was centrifuged at 14000 g for 10 min at 4 °C and the clear supernatant served as crude enzyme source. Crude enzyme was added to 0.5 ml of 1 % CMC in 0.05 M phosphate buffer and incubated at 50 °C for 30 min. After incubation, reaction was stopped by the addition of 1.5 ml of DNS reagent and boiled at 100 °C in water bath for 10 min. Sugars liberated were determined by measuring absorbance at 540 nm. Cellulase production was estimated by using glucose calibration curve. One unit (U) of enzyme activity is expressed as the quantity of enzyme, which is required to release 1 μ mol of glucose per minute under standard assay conditions.

Table 3.6.1 **Experiment: 1**

Details of the treatments	
(Agricultural waste as substrate)	
Treatments	Substrates
T ₁	Composting without pretreatment
T ₂	Composting with pretreatment
T ₃	Vermicomposting without pretreatment
T ₄	Vermicomposting with pretreatment
T ₅	Bio ethanol production without pretreatment
T ₆	Bio ethanol production with pretreatment
T ₇	Biogas production without pretreatment
T ₈	Biogas production with pretreatment

Table 3.6.2 **Experiment: 2**

Details of the treatments	
(Horticultural waste as substrate)	
Treatments	Substrates
T ₁	Composting without pretreatment
T ₂	Composting with pretreatment
T ₃	Vermicomposting without pretreatment
T ₄	Vermicomposting with pretreatment
T ₅	Bio ethanol production without pretreatment
T ₆	Bio ethanol production with pretreatment
T ₇	Biogas production without pretreatment
T ₈	Biogas production with pretreatment

3.6 LABORATORY ANALYSIS

Analysis of biogas slurry samples

3.6.1 Total solids percentage (TS %) in the slurry samples

Total solids % in the slurry samples were determined by drying a 100 g of sample for 105 °C for 24 h (APHA, 1992).

$$\text{Total solids} = \frac{\{(\text{Dry weight of the sample} + \text{weight of crucible}) - (\text{weight of the crucible})\}}{\text{fresh weight of the sample}} \times 100$$

3.6.2 Total volatile solids percentage (%) in the slurry samples (TVS)

Total volatile solids % in the slurry samples were determined by incinerating the dry sample at 550 °C for 2 h (APHA, 1992).

Total volatile solids =

$$\frac{\{(\text{Dry weight of the sample} + \text{weight of crucible}) - (\text{weight of the crucible weight of sample after ignition})\}}{(\text{fresh weight of the sample} + \text{weight of crucible}) - (\text{weight of the crucible})} \times 100$$

3.6.3 Volatile fatty acids in the slurry samples (VFA)

Volatile fatty acids in the slurry samples were estimated by titration method (APHA, 1992).

3.6.4 pH in the slurry samples

pH of the slurry samples were determined in 1:2.5 substrate:water suspension by using digital pH meter (Systronics μ pH system361) (Jackson, 1973).

3.6.5 Total N, P, K content in the slurry samples

3.6.5.1 Total Nitrogen content

Total nitrogen in slurry samples were estimated by modified Kjeldahl method using sulphuric and salicylic acid mixture. One gram of slurry sample was taken into 100 ml conical flask, 30 ml of sulphuric acid - salicylic acid mixture and 0.5 g of sodium thio-sulphate was added mixed well and kept aside for half an hour and digested on flame. After 30 min of digestion, one gram of copper sulphate and 10 g of potassium sulphate was added, digestion was continued till colourless solution obtained. The digested material was washed with distilled water and only supernatant liquid was transferred to a beaker. From that beaker solution was transferred to kjeldhal flask. 50 ml of 4 % boric acid taken into 250 ml conical flask to which two drops of mixed indicator was added and kept at the flask at the receiving end of distillation set in such a way that the receiving end immersed into the solution. Few Zn pieces, little quantity of paraffin and 120 ml of 40 % NaOH was added to the Kjeldhal flask and immediately mouth of the flask was closed. The distillation continued till no more ammonia was evolved at the receiving end of the distillation set. At the end of distillation, the tip of receiving end was washed with distilled water, contents of the flask were cooled and titrated against 0.01 N H_2SO_4 till blue colour changed to pinkish red colour.

Burette reading was noted and nitrogen % was calculated as:

Weight of the plant sample taken = 0.1 g

Blank titre value = B ml of 0.01 N H_2SO_4

Sample titre value = S ml of 0.01 N H_2SO_4

Actual titre value = (S - B) ml

$$1000 \text{ ml of } 1 \text{ N H}_2\text{SO}_4 = 14 \text{ g N}$$

$$(S - B) \text{ ml of } 0.01 \text{ N H}_2\text{SO}_4 = \frac{(S-B)0.01 \times 14}{1000}$$

Present in 0.1 g plant sample

$$100 \text{ g of plant sample contains} = \frac{(S-B) 0.01 \times 14 \times 100 \text{ g of N}}{1000 \times 0.1}$$

$$= (S - B) \times 0.14 \% \text{ of N}$$

3.6.5.2 Total Phosphorus content

Total phosphorus content in slurry samples were determined by perchloric acid digestion method using Barton's reagent as described by Jackson (1967). One gram of slurry sample was taken into 100 ml conical flask and 12-15 ml of tri acid mixture was added (Nitric acid: Sulphuric acid: Perchloric acid at 9:2:1). The mouth of the flask was covered with a funnel. The contents were digested over a sand bath till clear solution was obtained. The filtrate was collected and 5 ml was taken into 25 ml volumetric flask and 5 ml of Barton's reagent was added and volume made up to 25 ml with distilled water. Yellow colour was developed in 30 minutes and intensity of colour was measured in a photoelectric colorimeter using blue filter (470 nm). The colour will be stable for 24 h. Standard curve was prepared and the concentration of phosphorus in the solution was deduced from that value and the percentage of phosphorus in the sample was calculated.

Concentration of phosphorus in coloured solution = X ppm

i.e., 1 ml of coloured solution contains = X µg P

50 ml of coloured solution contains = 50 × X µg P

Which is present in 5 ml of the diluted digest

100 ml of diluted plant digest contains of $50 \times X \times \frac{100}{5} = X \times 1000 \text{ µg P}$

Which is obtained from 1 g sample

100 g of sample consists of = $X \times 1000 \times \frac{100}{1}$

= $X \times 10^5 \times 10^{-6} \% \text{ of P}$

= X × 0.1 %

3.6.5.3 Total Potassium content

Tri-acid extract was directly aspirated to the flame photometer to estimate the total potassium content (Systronics flame photometer 128) by Jackson (1967). 5 ml of tri-acid extract was taken into 25 ml volumetric flask and volume made upto the mark with distilled water. The concentration of K in the solution was measured using flame photometer. Standard curve was prepared and the concentration of K in the solution was deduced from that value and the percentage of K in the sample was calculated. Amount of K present in the sample (% of K) =

Concentration of K in the sample = X ppm

1 ml of the sample = X µg of K

100 ml of the sample = ?

$$= 100 \times X \text{ µg of K}$$

1 g of sample = 100 × X µg of K

100 g of sample = ?

$$= \frac{100}{1} \times 100 \times X \text{ µg of K}$$

$$= X \times 10^4 \times 10^{-6} \text{ g K}$$

$$= X \times 0.01 \%$$

3.6.6 Total Organic Carbon content

Organic carbon content of the slurry sample was estimated by Walkley and Black's wet oxidation method as outlined by Walkley and Blacks (1934). One gram of slurry sample was taken 500 ml conical flask and to it 10 ml of 1 N K₂Cr₂O₇ and 20 ml of Conc. H₂SO₄ was added. Diphenylamine indicator was added and titrated with 0.5 N ferrous ammonium sulphate solution until green colour appearing. A blank was run along with the sample.

Organic carbon % in slurry sample =

$$B \times 0.003 \times \frac{10(B - S)}{\text{weight of the sample taken (g)}}$$

Titre value of the blank in ml = B

Titre value of the sample in ml = S

3.6.7 Biological Oxygen Demand (BOD)

Biological Oxygen Demand was determined by measuring oxygen concentration in slurry sample, idometrically before and after incubation in the dark at 20 °C for 5 days by the colorimetric method (APHA, 1992). BOD bottles were taken and filled with the sample and stopper was placed. Then the stopper was removed and 1 ml of each manganous sulphate and alkaline potassium sulphate were added. Precipitation was dissolved by adding 2 ml of sulphuric acid. Then the whole content was transferred into a conical flask. Few drops of starch indicator was added and titrated against sodium thiosulphate solution till the initial blue colour turned to colourless. The titre value obtained gives dissolved Oxygen (D.O₁)

$$\text{Dissolved Oxygen (D.O}_1\text{)} = \frac{(V_1N \times 8 \times 1000)}{V_2 - V_3}$$

Volume of titrant (ml) = V₁

Volume of sampling bottle after placing the stopper (ml) = V₂

Volume of manganous sulphate and potassium sulphate solution added (ml) = V₃

Other set of BOD bottles were prepared same as above and incubated in BOD incubator for 5 days. After 5 days the content from BOD bottles were transferred into conical flasks and titrated similarly. The dissolved oxygen content D.O₅ was determined.

BOD = (D.O₁ - D.O₅) × dilution factor.

3.6.8 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand was determined by taking centrifuged slurry samples and refluxed with a known amount of Potassium dichromate in sulphuric acid medium and excess of dichromate was titrated against ferrous ammonium sulphate. The amount of dichromate consumed was proportional to the oxygen required to oxidize the organic matter. Chemical oxygen demand was determined by the closed reflux colorimetric method (APHA, 1992).

$$\text{COD} = \frac{(B - T) \times N \times 1000 \times 8}{\text{Volume of sample (ml)}}$$

3.6.9 Estimation of cellulose

Cellulose content was estimated by the method of Uppdegraff (1969). Hundred milligram of pretreated cellulose wastes were added with 5 ml of Nitric reagent and boiled and cooled. It was centrifuged at 5000 rpm for 5 min. The pellet was washed with distilled water. 10 ml of 67 % sulphuric acid was added. One ml of the sample was diluted to 100 ml. To 1 ml of the each diluted solution, 10 ml of freshly prepared ice cooled anthrone reagent was added and boiled in a boiling water bath for 10 min at 100 °C. After that absorbance was recorded at 600 nm.

3.7 Measurement of Gas production

The biogas production readings were taken on an alternate day by water displacement method with the measuring jar.

3.7.1 Estimation of methane percentage using gas chromatography

Methane percentage in the biogas was estimated by using gas chromatography (Bruker-450) with a Flame Ionization Detector (FID) temperatures were maintained at 300 °C in the detector, 75 °C in the injector and 50 °C in the oven. The column used was porapak Q. The gas flow in the column was maintained as 60 ml min⁻¹.

3.7.2 Bio ethanol production

The 200 ml of substrate with medium was taken into the bottles for fermentative production of alcohol. All the bottles were pasteurized at 82 °C for 30 minutes and rapidly cooled at room temperature. The culture inoculum were transferred to the pasteurized fermentation bottles containing 200 ml of sucrose medium, 2 g of yeast extract powder and 100 ppm of potassium metabisulphite (K₂S₂O₅) at the rate of 5 % under aseptic conditions.

3.7.3 Ethanol estimation

One ml of the fermented wash was taken in 500 ml volumetric flask containing 30 ml of distilled water. The distillate was collected in 50 ml flask containing 25 ml of potassium dichromate solution (33.76 g of K₂Cr₂O₇ dissolved in 400 ml of distilled water with 325 ml of sulphuric acid and volume raised to 1 litre). About 20 ml of distillate was collected in each sample and the flasks were kept in a water bath maintained at 62.5 °C for 20 min. The flasks were cooled to room temperature and the volume raised to 50 ml.

5 ml of this was diluted with 5 ml of distilled water for measuring the optical density at 600 nm using spectrophotometer.

3.8 Statistical Analysis

The analysis and interpretation of data were done using the Fischer's method of analysis of variance technique as described by Gomez and Gomez (1984). The levels of significance used in 'F' and 't' test was $P = 0.05$ and critical values were calculated wherever the 'F' test was significant.

Chapter IV

RESULTS AND DISCUSSION

The results of the experiment entitled “**AEROBIC AND ANAEROBIC DIGESTION OF AGRICULTURAL WASTE FOLLOWED BY VERMICOMPOSTING AND ENRICHMENT**” conducted during 2015-16 in the Department of Agricultural Microbiology and Bioenergy, College of Agriculture, Rajendranagar, PJTSAU, are presented in this chapter.

Agricultural and horticultural wastes were digested under aerobic condition by composting and vermicomposting and under anaerobic condition by fermentation to produce bioethanol and biogas. The wastes were enriched with efficient cellulose degrading bacterial culture as part of pretreatment.

4.1 COLLECTION OF DIFFERENT SAMPLES FOR ISOLATION OF CELLULASE DEGRADING BACTERIA

4.1.1 Soil Sample Collection

Soil samples were collected from agricultural college, student farm, farmer's fields, forest soil and another sample was collected from horse dung dump near veterinary college. These soil samples were air dried and mixed thoroughly and sieved through a 2 mm sieve.

4.1.2 Waste Collection

Samples were collected from waste receiving dustbin of Domestic kitchen wastes, Municipal solid waste, Sewage water and cow dung in pre-sterilized screw cap glass vials of 30 ml capacity.

4.1.3 Isolation of Cellulose Degrading Bacteria

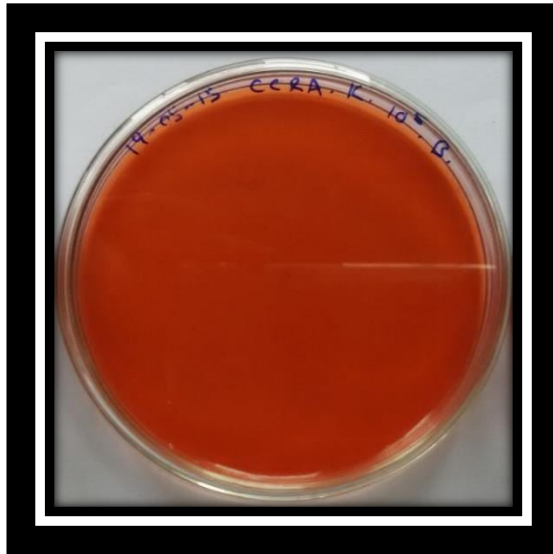
Cellulose degrading bacteria was isolated from different soil samples and waste samples [Cow Dung Sample (CDS), Municipal Solid Waste (MSW), Domestic Kitchen Waste (DKW) and Sewage Water Sample (SWS)] by using CMC agar medium. Total number of cellulose degrading bacterial colonies were counted. Among all the samples SWS showed more no. of colonies (145×10^6 CFU g⁻¹) on CMC agar medium followed by HDD (145×10^6 CFU g⁻¹), FF (110×10^6 CFU g⁻¹) and lowest colony count was

Table 4.1. Isolation of cellulose degrading bacteria

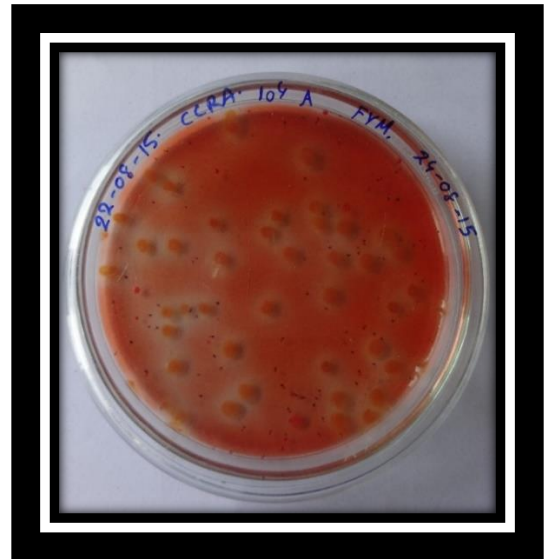
Samples	(10⁶ CFU g⁻¹)
CDS	64
MSW	97
DKW	52
SWS	155
FF	110
HDD	145
C.D. (P=0.05)	1.799
SE(m)	0.577
C.V.	0.965

CDS- Cow dung from cattle shed at Rajendranagar; MSW- Municipal solid waste from garbage disposal place of Rajendranagar; DKW- Domestic kitchen waste from hostel mess; SWS- Sewage water sample from sewage sludge of Rajendranagar; FF-Farmers' Fields of college farm at Rajendranagar; HDD-Horse dung dump near veterinary college.

Plate 4.1 Isolation of cellulose degrading bacterial isolates

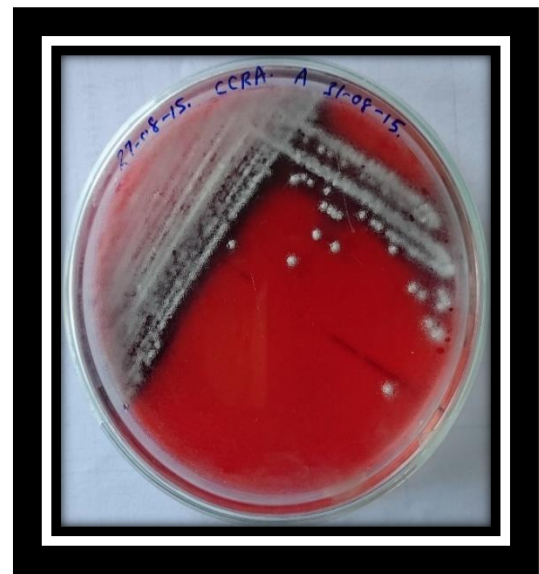


CCRA- Control plate



CCRA- Plate with colonies

Plate 4.2 Screening of cellulose degrading bacterial isolates



CCRA- Media plates with cellulose degrading bacterial colonies

observed in DKW (52×10^6 CFU g⁻¹). Thirty morphologically distinct bacterial isolates which were giving halo zones in CMC agar medium were selected and named as CDB-1, CDB-4, CDB-5, CDB-7, CDB-9 etc (Table 4.1). Gautam *et al.* (2012) studied diversity of cellulolytic microbes and biodegradation of municipal solid waste by a potential strain.

4.1.4 Morphological Characterization of Different Isolates

The bacterial isolates were presumptively characterized by means of morphological characteristics (Table 4.1.1.A).

4.1.5 Biochemical Characterization

After the morphological characterization, the isolates were tested for different biochemical characters like catalase and urease production, IMVIC (Indole test, Methyl red reaction, Voges-Proskauer reaction, Citrate utilization), Nitrate reduction, Carbohydrate fermentation (acid-gas production), Starch hydrolysis, Gelatin liquefaction and Casein hydrolysis (Table 4.1.1.B).

The biochemical test were conducted for all 70 bacterial isolates 27 isolates were positive for indole reaction, 60 isolates positive catalase activity, all isolates were positive for oxidase activity, 61 isolates were positive for gelatine liquefaction, 31 isolates were positive for Voges Proskauer test, 57 isolates were positive for citrate utilization, 25 isolates were positive for starch hydrolysis, 35 isolates were positive for H₂S production, and in carbohydrate utilization tests 39 isolates utilized glucose, 37 isolates utilized galactose and 35 isolates used lactose. All isolates exhibited denitrification activity.

The isolates were identified based on gram reaction and colony morphology on different media. Seventy isolates showed growth on specialized media. Observations recorded after streaking and incubation on different medium. Both morphological, cultural biochemical characters were used for identification of the isolates using the criteria mentioned in Bergey's Manual of Systematic Bacteriology (Holt *et al.*, 1984).

4.1.6 Determination of Cellulase Activity of Bacterial Isolates

The clearing zone size and colony diameter of these isolates were measured when incubated aerobically at 37 °C; the result showed that maximum clearing zone ranged from 1.38 and 1.25 mm demonstrating that the isolates have the ability to degrade the carboxymethyl cellulose and indicating high ability of cellulase production. Among 30

Plate: 4.3 Biochemical characters of the cellulose degrading isolates



Indole test



Voges proskauer test



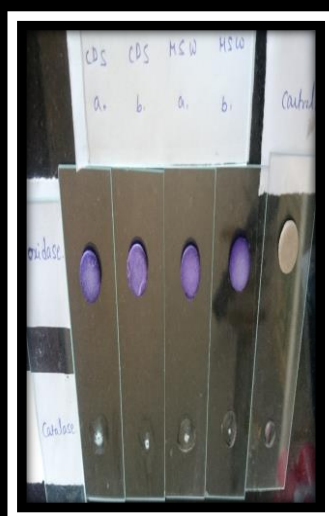
Methyl red test



Citrate utilization test



Gelatin liquefaction



Oxidase and catalase test



Starch hydrolysis

Table: 4.1.1.A Morphological characteristics of cellulose degrading bacteria

Isolate	Shape	Size	Gram reaction	Isolate	Shape	Size	Gram reaction
CDB-1	Rod	Small	Negative	CDB-36	Rod	Small	Negative
CDB-2	Rod	Medium	Negative	CDB-37	Rod	Small	Negative
CDB-3	Cocci	Small	Negative	CDB-38	Rod	Small	Negative
CDB-4	Rod	Small	Negative	CDB-39	Rod	Small	Negative
CDB-5	Cocci	Medium	Negative	CDB-40	Rod	Small	Negative
CDB-6	Rod	Small	Negative	CDB-41	Rod	Small	Negative
CDB-7	Rod	Small	Negative	CDB-42	Rod	Small	Negative
CDB-8	Rod	Small	Negative	CDB-43	Rod	Small	Negative
CDB-9	Rod	Medium	Negative	CDB-44	Cocci	Small	Negative
CDB-10	Cocci	Small	Negative	CDB-45	Rod	Small	Negative
CDB-11	Rod	Small	Negative	CDB-46	Rod	Medium	Negative
CDB-12	Rod	Small	Negative	CDB-47	Cocci	Small	Negative
CDB-13	Rod	Small	Negative	CDB-48	Rod	Medium	Negative
CDB-14	Cocci	Small	Negative	CDB-49	Rod	Small	Negative
CDB-15	Rod	Small	Negative	CDB-50	Rod	Small	Negative
CDB-16	Rod	Medium	Negative	CDB-51	Cocci	Small	Negative
CDB-17	Rod	Medium	Negative	CDB-52	Cocci	Small	Negative
CDB-18	Cocci	Small	Negative	CDB-53	Rod	Medium	Negative
CDB-19	Cocci	Small	Negative	CDB-54	Rod	Small	Negative
CDB-20	Cocci	Small	Negative	CDB-55	Rod	Small	Negative
CDB-21	Rod	Small	Negative	CDB-56	cocci	Small	Negative
CDB-22	Rod	Medium	Negative	CDB-57	Rod	Small	Negative
CDB-23	Rod	Small	Negative	CDB-58	Rod	Medium	Negative
CDB-24	rod	small	Negative	CDB-59	Cocci	Small	Negative
CDB-25	Cocci	Small	Negative	CDB-60	Rod	Medium	Negative

CDB-26	Rod	Medium	Negative	CDB-61	Rod	Medium	Negative
CDB-27	Cocci	Small	Negative	CDB-62	Cocci	Small	Negative
CDB-28	Rod	Medium	Negative	CDB-63	Cocci	Medium	Negative
CDB-29	Rod	Small	Negative	CDB-64	Rod	Small	Negative
CDB-30	Cocci	Small	Negative	CDB-65	Rod	Medium	Negative
CDB-31	Rod	Small	Negative	CDB-66	Rod	Medium	Negative
CDB-32	Rod	Small	Negative	CDB-67	Rod	Medium	Negative
CDB-33	Cocci	Small	Negative	CDB-68	Cocci	Small	Negative
CDB-34	Rod	Small	Negative	CDB-69	Cocci	Small	Negative
CDB-35	Rod	Small	Negative	CDB-70	cocci	Small	Negative

Table 4.1.1.B. Biochemical characteristics of cellulose degrading bacterial isolates

Characters	Biochemical tests	CDB-1	CDB-2	CDB-3	CDB-4	CDB-5	CDB-6	CDB-7	CDB-8	CDB-9	CDB-10	CDB-11	CDB-12
1	Indole test	-	-	+	-	+	-	-	-	+	+	-	-
2	Catalase test	+	-	+	+	-	-	+	+	-	+	-	+
3	Oxidase test	+	+	+	+	+	+	+	+	+	+	+	+
4	Gelatin liquifaction	-	-	-	+	+	+	+	+	-	+	+	+
5	Methyl red test	-	+	-	-	-	-	-	-	+	-	-	-
6	V-P test	+	+	+	+	+	+	+	+	+	+	+	+
7	Citraye utilization	-	+	+	+	-	+	+	+	+	+	+	+
8	Starch hydrolysis	-	-	-	+	-	-	+	+	+	-	-	-
9	H₂S	+	-	-	+	+	-	+	-	+	+	+	+
10	Denitrification	+	+	+	+	+	+	+	+	+	+	+	+
11	Glucose	+	+	-	-	-	+	+	-	+	+	-	+
12	Galactose	-	+	+	-	+	-	+	+	-	+	-	+
13	Lactose	-	-	-	+	-	-	+	+	+	-	-	-

Table 4.1.1. (cont.)

Table 4.1.1. (cont.)

S.No	Biochemical tests	CDB-13	CDB-14	CDB-15	CDB-16	CDB-17	CDB-18	CDB-19	CDB-20	CDB-21	CDB-22	CDB-23	CDB-24
1	Indole test	-	-	-	-	-	-	-	-	-	-	-	-
2	Catalase test	+	+	+	+	+	+	+	+	+	+	+	+
3	Oxidase test	+	+	+	+	+	+	+	+	+	+	+	+
4	Gelatin liquifaction	+	+	+	+	+	+	+	+	+	+	+	+
5	Methyl red test	-	-	-	-	-	-	+	-	-	-	-	-
6	VP test	-	+	+	-	+	+	+	+	+	+	-	+
7	Citrate utilization	-	+	+	+	+	+	-	+	-	+	+	+
8	Starch hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-
9	H ₂ S	+	-	+	-	-	+	+	+	+	-	+	-
10	Denitrification	+	+	+	+	+	+	+	+	+	+	+	+
11	Glucose	-	+	+	-	+	-	+	-	+	-	+	+
12	Galactose	+	+	-	+	+	+	-	-	+	-	-	+
13	Lactose	-	-	+	-	+	-	-	+	+	+	+	-

S.No	Biochemical tests	CDB-25	CDB-26	CDB-27	CDB-28	CDB-29	CDB-30	CDB-31	CDB-32	CDB-33	CDB-34
1	Indole test	-	-	-	-	-	-	-	-	-	-
2	Catalase test	+	+	+	+	+	+	+	+	+	+
3	Oxidase test	+	+	+	+	+	+	+	+	+	+
4	Gelatin liquifaction	+	+	+	+	+	+	+	+	+	+
5	Methyl red test	-	-	-	-	-	-	-	-	-	-
6	VP test	+	+	+	+	+	+	+	+	+	+
7	Citrate utilization	+	+	+	-	+	+	-	-	-	+
8	Starch hydrolysis	-	-	-	-	-	+	+	-	-	-
9	H ₂ S	-	+	-	+	-	+	-	+	-	+
10	Denitrification	+	+	+	+	+	+	+	+	+	+
11	Glucose	-	+	+	+	-	+	-	+	-	-
12	Galactose	+	-	+	-	-	-	+	-	+	+
13	Lactose	+	-	-	+	+	+	+	+	-	-

Table 4.1.1. (cont.)

S.No	Biochemical tests	CDB-35	CDB-36	CDB-37	CDB-38	CDB-39	CDB-40	CDB-41	CDB-42
1	Indole test	+	+	-	+	+	+	+	+
2	Catalase test	-	+	+	+	+	+	+	+
3	Oxidase test	+	+	+	+	+	+	+	+
4	Gelatin liquifaction	+	+	+	+	+	+	+	+
5	Methyl red test	+	+	+	+	+	+	+	+
6	VP test	-	-	-	-	-	-	-	-
7	Citrate utilization	-	+	+	+	-	+	+	+
8	Starch hydrolysis	-	+	-	+	+	+	+	-
9	H ₂ S	+	-	-	+	-	-	-	+
10	Denitrification	+	+	+	+	+	+	+	+
11	Glucose	-	+	-	+	+	-	+	-
12	Galactose	-	-	+	-	-	+	-	+
13	Lactose	-	+	+	-	+	+	-	+

Table 4.1.1. (cont.)

S.No	Biochemical tests	CDB-43	CDB-44	CDB-45	CDB-46	CDB-47	CDB-48	CDB-49	CDB-50	CDB-51	CDB-52	CDB-53	CDB-54
1	Indole production	+	-	-	+	+	+	-	-	-	-	+	-
2	Catalase test	+	+	+	+	+	+	+	+	+	+	-	+
3	Oxidase test	+	+	+	+	+	+	+	+	+	+	+	+
4	Gelatin liquefaction	+	+	+	+	-	+	-	+	+	+	+	+
5	Methyl red test	+	+	-	-	+	+	+	+	-	-	-	-
6	VP test	-	-	-	-	-	-	-	-	-	-	-	-
7	Citrate utilization	-	+	+	-	+	+	+	+	+	+	+	+
8	Starch hydrolysis	+	-	-	+	-	+	-	+	-	+	-	+
9	H ₂ S	+	-	-	+	-	-	+	+	-	+	+	-
10	Denitrification	+	+	+	+	+	+	+	+	+	+	+	+
11	Glucose	-	+	-	+	-	+	+	-	-	+	-	+
12	Galactose	+	+	-	+	+	-	+	-	+	-	+	+
13	Lactose	-	-	+	+	+	-	-	-	-	+	-	-

Table 4.1.1. (cont.)

S.No	Biochemical tests	CDB -55	CDB -56	CDB -57	CDB -58	CDB -59	CDB -60	CDB -61	CDB -62	CDB -63	CDB -64	CDB -65	CDB -66	CDB -67	CDB -68	CDB -69	CDB -70
1	Indole production	-	+	-	+	+	+	+	+	-	+	+	-	-	+	+	+
2	Catalase test	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	+
3	Oxidase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Gelatin liquifaction	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+
5	Methyl red test	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
6	VP test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Starch hydrolysis	-	-	-	-	+	+	+	+	-	-	+	-	-	+	+	+
9	H ₂ S	+	-	+	-	+	-	-	+	-	+	-	-	-	-	-	+
10	Denitrification	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	Glucose	+	+	-	+	+	-	+	-	+	+	+	+	-	-	+	-

12	Galactose	-	-	+	-	-	+	+	-	-	+	-	-	+	-	+	+
13	Lactose	+	+	-	+	+	-	-	+	+	-	+	-	-	+	+	+

bacterial isolates, the isolates CDB-30 and CDB-34 as evidenced by its maximum clearing zone value of 1.38 mm, followed by CDB-43 (1.32 mm), CDB-36 (1.30 mm), CDB-10 (1.25 mm), CDB-64 (1.25 mm), CDB-11, CDB-26, CDB-41, CDB-31 (1.18 mm), CDB-15 (1.13 mm). Similarly quantitative estimation of cellulose activity results revealed that same manner (CDB-30: 0.149 mg ml⁻¹, CDB-34: 0.148 mg ml⁻¹, CDB-10: 0.136 mg ml⁻¹, CDB-36: 0.135 mg ml⁻¹, CDB-43: 0.129 mg ml⁻¹) (Table 4.1.2 and 4.1.3) Hatami *et al.* (2008) reported the ratio of the zone diameter to colony diameter ratio was 0.4 to 2.1.

4.2 ANALYSIS

4.2.1 Chemical Composition of Agricultural Waste

The percentage of total solids in the Agricultural waste (maize straw) showed in the Table 4.2 revealed that the percentage of total solids were significantly more in T₁ (Composting without pretreatment) 19.46 %, followed by T₂ (Composting with pretreatment) 18.96 %, T₃ (Vermicomposting without pretreatment) 15.26 %, T₄ (Vermicomposting with pretreatment) 14.65 % and low percentage of total solids were recorded in T₅ (Biogas production without pretreatment) 7.00 per cent. Followed by the percentage of total volatile solids in T₂ (Composting with pretreatment) (80.46 %) followed by T₅ (Biogas production without pretreatment) (80.20 %), T₃ (Vermicomposting without pretreatment) (79.86 %), T₆ (Biogas production with pretreatment) (79.50 %) and low amount was observed in T₁ (Composting without pretreatment) (70.58 %). The concentration of volatile fatty acids were significantly more in T₁ (Composting without pretreatment) (0.95 g l⁻¹) followed by T₂ (Composting with pretreatment) (0.89 g l⁻¹), T₃ (Vermicomposting without pretreatment) (0.76 g l⁻¹), T₅ (Biogas production without pretreatment) (0.70 g l⁻¹) and low in T₆ (Biogas production with pretreatment) (0.60 g l⁻¹). The pH of all treatments were recorded. The maximum pH was observed as slightly alkaline in T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment), T₄ (Vermicomposting with pretreatment) 8.2 followed by T₁ (Composting without pretreatment) (8.0) and acidic pH was recorded in T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) (6.2). The percentage of nitrogen content in the agricultural waste was significantly more in T₄ (Vermicomposting with pretreatment) (1.84 %) followed T₅ (Biogas production without pretreatment) (1.68 %), T₆ () (1.50 %) and low value was recorded in T₁ (Composting without pretreatment) (0.60 %). The percentage of

Table 4.1.2 Cellulase activity of bacterial isolates

Source-1							
S.No	Isolates	CDS	MSW	DKW	SWS	Zone (mm)	Enzyme activity (mg/ml)
1	CDB-1	+	-	-	-	0.75	0.032
2	CDB-4	-	+	-	-	0.35	0.020
3	CDB-5	-	+	-	-	0.20	0.016
4	CDB-7	-	-	+	-	0.55	0.015
5	CDB-9	-	-	+	-	0.53	0.015
6	CDB-10	-	-	-	+	1.25	0.136
7	CDB-11	-	-	-	+	1.18	0.126
8	CDB-15	-	-	-	+	1.13	0.124
9	CDB-18	-	-	-	-	0.25	0.018
10	CDB-21	-	+	-	-	0.98	0.019
11	CDB-23	-	+	-	-	0.75	0.020
12	CDB-24	-	-	+	-	0.93	0.013
13	CDB-26	-	-	+	-	1.18	0.125
14	CDB-28	-	-	-	+	0.98	0.024
15	CDB-30	-	-	-	+	1.38	0.149
	C.D.					0.058	0.005
	SE(m)					0.020	0.002
	C.V.					4.225	5.493

CDS- Cow dung sample; MSW- Municipal solid waste; DKW- Domestic kitchen waste; SWS- Sewage water sample.

+ and – indicate : Source of sample for isolation of bacterial isolates

Table 4.1.3 Cellulase activity of bacterial isolates

Source-2								
S.No	Isolates	FF	HDD	MSW	DKW	SWS	Zone (mm)	Enzyme activity (mg/ml)
1	CDB-31	+	-	-	-	-	1.18	0.126
2	CDB-34	+	-	-	-	-	1.38	0.148
3	CDB-35	+	-	-	-	-	0.98	0.015
4	CDB-36	+	-	-	-	-	1.30	0.135
5	CDB-38	+	-	-	-	-	0.95	0.014
6	CDB-41	+	-	-	-	-	1.18	0.131
7	CDB-43	+	-	-	-	-	1.32	0.129
8	CDB-44	-	+	-	-	-	0.93	0.032
9	CDB-50	-	+	-	-	-	0.98	0.046
10	CDB-54	-	+	-	-	-	0.85	0.048
11	CDB-56	-	+	-	-	-	0.95	0.023
12	CDB-60	-	+	-	-	-	0.86	0.047
13	CDB-62	-	-	+	-	-	0.53	0.026
14	CDB-64	-	-	-	+	-	1.25	0.136
15	CDB-67	-	-	-	-	+	0.70	0.042
	C.D.						0.052	0.004
	SE(m)						0.018	0.002
	C.V.						3.050	3.580

MSW- Municipal solid waste; DKW- Domestic kitchen waste; SWS- Sewage water sample.

FF-Farmers' Fields; HDD-Horse dung dump

+ and – indicate : Source of sample for isolation of bacterial isolates

phosphorus content in the agricultural waste was significantly more in T₅ (Biogas production without pretreatment) (1.50 %) followed by T₆ (Biogas production with pretreatment) (1.30 %), T₃ (Vermicomposting without pretreatment), T₅ (Biogas production without pretreatment) (1.00 %) and least recorded in T₁ (Composting without pretreatment) (0.15 %). The percentage of potassium content in the agricultural waste was significantly more in T₅ (Biogas production without pretreatment) (1.09 %) followed by T₄ (Vermicomposting with pretreatment), T₆ (Biogas production with pretreatment) (1.00 %), T₃ (Vermicomposting without pretreatment) (0.99 %) and low amount was recorded in T₁ (Composting without pretreatment) (0.65 %). The percentage of organic carbon content in the agricultural waste was significantly more in T₆ (Biogas production with pretreatment) (45.60 %) followed by T₅ (Biogas production without pretreatment) (33.40 %), T₁ (Composting without pretreatment) (25.50 %), T₂ (Composting with pretreatment) (24.00 %) and low amount was recorded in T₃ (Vermicomposting without pretreatment) (21.60 %). The concentration of biological oxygen demand in the agricultural waste was significantly more in T₄ (Vermicomposting with pretreatment) (98.30 mg l⁻¹) followed by T₆ (Biogas production with pretreatment) (96.75 mg l⁻¹), T₃ (Vermicomposting without pretreatment) (95.40 mg l⁻¹), T₅ (Biogas production without pretreatment) (95.20 mg l⁻¹) and least recorded in T₁ (Composting without pretreatment) (92.00 mg l⁻¹). The concentration of chemical oxygen demand in the agricultural waste was significantly more in T₄ (Vermicomposting with pretreatment) (120.40 g l⁻¹) followed by T₃ (Vermicomposting without pretreatment) (110.20 g l⁻¹), T₆ (Biogas production with pretreatment) (101.60 g l⁻¹) and least recorded in T₂ (Composting with pretreatment) (95.00 g l⁻¹). The percentage of cellulose content in the agricultural waste was significantly more in T₂ (Composting with pretreatment) (27.58 %) followed by T₄ (Vermicomposting with pretreatment) (26.10 %), T₆ (Biogas production with pretreatment) (25.89 %), T₅ (Biogas production without pretreatment) (25.33 %) and low amount was recorded in T₁ (Composting without pretreatment) (24.31 %) (Table 4.2).

The results of microbial counts in substrates (Agricultural Waste) revealed that, the treatments T₁ (Composting without pretreatment) observed maximum bacterial counts (9.9×10^6 CFU g⁻¹) followed by T₂ (Composting with pretreatment) (6.9×10^6 CFU g⁻¹), T₄ (Vermicomposting with pretreatment) (4.8×10^6 CFU g⁻¹), T₅ (Biogas production without pretreatment) (4.6×10^6 CFU g⁻¹) and low bacterial counts were observed in treatment T₆ (Biogas production with pretreatment) (3.2×10^6 CFU g⁻¹). In case of fungal counts maximum fungal count was recorded in T₆ (Biogas production with pretreatment) (4.4×10^4 CFU g⁻¹) followed by T₅ (Biogas production without pretreatment) (4.2×10^4

Table 4.2. Chemical composition of different substrates at initial stage used in the treatments of Agricultural waste

Agricultural Waste	Total solids % (TS)	Total volatile solids % (TVS)	Volatile fatty acids g l⁻¹ (VFA)	pH	N %	P %	K %	Organic Carbon %	BOD mg l⁻¹	COD g l⁻¹	Cellulose %
T₁	19.46	70.58	0.95	8.00	0.60	0.15	0.65	25.50	92.00	98.80	24.31
T₂	18.96	80.46	0.89	8.20	0.70	0.16	0.70	24.00	93.50	95.00	27.58
T₃	15.26	79.86	0.76	8.25	1.65	1.00	0.99	21.60	95.40	110.20	24.79
T₄	14.65	71.85	0.68	8.20	1.84	1.00	1.00	22.60	98.30	120.40	26.10
T₅	7.00	80.20	0.70	6.20	1.68	1.50	1.09	33.40	95.20	100.30	25.33
T₆	9.30	79.50	0.60	6.30	1.50	1.30	1.00	45.60	96.75	101.60	25.89
C.D.	1.033	4.168	0.028	0.535	0.079	0.037	0.037	2.125	3.426	3.769	1.814
SE(m)	0.332	1.338	0.009	0.172	0.025	0.012	0.012	0.682	1.100	1.210	0.582
C.V.	4.068	3.007	2.071	3.950	3.285	2.444	2.444	4.103	2.001	2.007	3.927

AW- Agricultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replication

CFU g⁻¹), T₃ (Vermicomposting without pretreatment) (3.6×10⁴ CFU g⁻¹) and low fungal count was recorded in T₁ (Composting without pretreatment) (2.3×10⁴ CFU g⁻¹). (Table 4.3).

The overall percentage of total solids in all the treatments were recorded highest in T₁ (Composting without pretreatment) (19.46 %), total volatile solids in T₂ (Composting with pretreatment) (80.46 %), volatile fatty acids in T₁ (Composting without pretreatment) (0.95 g l⁻¹), pH in T₃ (Vermicomposting without pretreatment) (8.25), N content in T₄ (Vermicomposting with pretreatment) (1.84 %), P content in T₅ (Biogas production without pretreatment) (1.50 %), K content in T₅ (Biogas production without pretreatment) (1.09 %), organic carbon content in T₆ (Biogas production with pretreatment) (45.60 %), biological oxygen demand in T₄ (Vermicomposting with pretreatment) (98.30 mg l⁻¹), chemical oxygen demand in T₄ (Vermicomposting with pretreatment) (120.40 g l⁻¹) and cellulose in T₂ (Composting with pretreatment) (27.58 %), bacterial population count were highest in T₁ (Composting without pretreatment) (9.9 x 10⁶ CFU g⁻¹), fungal population count was highest in T₆ (Biogas production with pretreatment) (4.4 x10⁴ CFU g⁻¹), actinomycetes population count were highest in T₁ (Composting without pretreatment) (9.8 x 10⁴ CFU g⁻¹) respectively (Table 4.3). The chemical composition in the treatments of compost, vermicompost and biogas production differed significantly. Nutrient content in different treatments depended mainly on substrate used, ratio of the dung and supplementing substrate used, maintenance of moisture in the treatments, environmental conditions and the time kept for running the experiment.

Composting of sugarcane waste by-products through treatment with microorganisms and subsequent vermicomposting resulted in N content 0.80 %, P content 0.53 % and K content 1.28 % results reported by Kumar *et al.* (2010).

The results of the present study is in agreement with the observation of Carey *et al.* (2016) who reported that during recovery of nutrients from bio refineries using combinations of physical, chemical, and biological operations and recorded N content 1.30 %, P content 0.21 % and K content 1.48 %.

Table 4.3 Population of bacteria, fungi and actinomycetes in agricultural waste

Agricultural waste			
RAW	Bacteria (10⁶ CFU g⁻¹)	Fungi (10⁴ CFU g⁻¹)	Actinomycetes (10⁴ CFU g⁻¹)
T₁	9.90	2.30	9.80
T₂	6.90	3.40	8.20
T₃	4.50	3.60	7.20
T₄	4.80	3.30	6.50
T₅	4.60	4.20	4.20
T₆	3.20	4.40	4.60
C.D	0.430	0.180	0.254
SE(m)	0.138	0.058	0.082
C.V	4.230	2.830	2.095

AW- Agricultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

4.2.2 Chemical Composition of Agricultural Waste Enriched With and Without Cellulose Degrading Bacteria

4.2.2.1 Contents of Total Solids (TS %), Total Volatile Solids (TVS %) and Volatile Fatty Acids (VFA)

Among the different treatments and intervals the TS % was found highest with the treatment T₁ (Composting without pretreatment). Among the different intervals the total solids % was low initially at 0th day and it was increased up to 14th day and later gradually decreased up to 28th day. Among all the treatments T₁ (Composting without pretreatment) recorded the highest i.e., 19.46 to 28.05 % followed by T₂ (Composting with pretreatment) (i.e., 18.96 to 25.49 %) and least was recorded in the treatment T₆ (Biogas production with pretreatment) (i.e., 7.24 to 9.30 %) (Table 4.4). In agricultural waste aerobic composting method TS % was more in composting with pretreatment of cellulose degrading bacteria compared to composting without pretreatment. Because of enriched compost containing less TVS % because of degradation of TS % in aerobic composting. When considering biogas production from a slurry, the VS content of the material is as important as the TS content, since it represents the fraction of the solid material that may be transformed into biogas. Most manure and sludge from municipal wastes have a VS content of 70-90 % of the TS content. The fixed solids (FS, also termed the ash content) is comprised of inorganic material which dilutes energy content and can impact the treatment process.

Among the different treatments and intervals the TVS found highest with the treatments T₂ (Composting with pretreatment). Among the different intervals the TVS % was increased up to 7th day. Among different treatments T₂ (Composting with pretreatment) recorded the highest TVS % i.e., 79.52 to 83.30 % and found on par with the treatments T₆ (Biogas production with pretreatment) and found lowest with the treatment T₁ (Composting without pretreatment) (Table 4.4).

These results are accordance with the findings of Barik *et al.* (2011) who reported that the Total Volatile Solids (TVS) per cent was 83.80 % were found in the vermicompost from agricultural wastes.

Although the VS content is an indicator of potential methane production, the specific methane yield on a VS basis is not a constant composition of the VS of the waste which includes both readily degradable organic compounds including lipids, proteins, and carbohydrates, as well as more refractory organics which may include lignocellulosic

materials, complex lipopolysaccharides, structural proteins (keratin) and other refractory organics. For pure carbohydrates the specific methane yield on VS basis is $0.38 \text{ m}^3 \text{ kg}^{-1}$ VS destroyed, for proteins the yield varies depending on amino acid profile and averages $0.6 \text{ m}^3 \text{ kg}^{-1}$ VS destroyed and for pure lipids (vegetable oil) the specific methane yield is $1.0 \text{ m}^3 \text{ kg}^{-1}$ VS destroyed. This difference can be attributed to the high H : C ratio of lipids compared to carbohydrates.

VFA % among all treatments and intervals were found highest with the treatment T₁ (Composting without pretreatment). Among all the intervals the VFA % was found to be increased up to 14th day and then gradually decreased up to 28th day. T₁ (Composting without pretreatment) (i.e. 0.84 to 0.96 g l^{-1}) showed the highest VFA % among all the treatments which was significant and followed by T₂ (Composting with pretreatment) (i.e. 0.68 to 0.91 g l^{-1}) and least VFA % was found with the treatments T₆ (Biogas production with pretreatment) (i.e. 0.60 to 0.78 g l^{-1}) (Table 4.4).

The fatty acids and alkalinity concentration showed fast changes when the stability of the anaerobic digestion process is upset. Because, when the process is not stable, the volatile fatty acids concentration increases, and the alkalinity decreases. The ratio of these two parameters can be a good indicator for the observation of the stability of the anaerobic digestion process. VS reduction, TS reduction, VS : TS ratio, COD reduction, pH and biogas production with its methane content. Although, variations in digester performance were observed in the early period of digestion, the observed pH of 6.65-7.81 were primarily within the acceptable range for anaerobic digestion for the entire operations. This implies average buffering capacity of the mixed substrate. Generally, degradation of substrates starts between day one to day three before it commences the production of biogas.

Similar observations have been made by earlier works of Barik *et al.* (2011) reported that increased N (0.51 and 1.61 %), P (0.19 and 1.02 %) and K (0.15 and 0.73 %) were found in the vermicompost from agricultural wastes.

4.2.2.2 pH

Among the different treatments the pH was found highest in T₃ (Vermicomposting without pretreatment) (8.25) on 0th day and the result was found on par with the treatments of T₁ (Composting without pretreatment), T₂ (Composting with pretreatment) and T₄ (Vermicomposting with pretreatment). Among the different

intervals the pH was slightly low initially and it was increased up to 21st day during 28th day slightly reduced in pH

Table 4.4. Total solids (%), total volatile solids (%) and volatile fatty acids content in agricultural waste at different intervals.

Agricultural waste	TS (%)					TVS (%)					VFS (g l ⁻¹)				
	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day
T₁	19.46	24.88 (5.42)	28.05 (3.17)	21.06 (6.99)	16.56 (4.50)	70.58	71.26 (0.68)	72.55 (1.29)	65.84 (6.71)	62.38 (3.46)	0.95	0.96 (0.01)	0.95 (0.05)	0.85 (0.06)	0.84 (0.01)
T₂	18.96	20.56 (1.60)	25.49 (4.93)	22.41 (3.08)	15.24 (7.17)	80.46	81.25 (0.79)	83.30 (2.05)	82.95 (0.35)	79.52 (3.43)	0.89	0.90 (0.01)	0.91 (0.05)	0.82 (0.13)	0.68 (0.14)
T₃	15.26	15.86 (0.60)	14.50 (1.36)	13.03 (1.47)	10.45 (2.58)	79.86	78.20 (1.66)	75.47 (2.73)	70.03 (5.44)	78.56 (8.53)	0.76	0.79 (0.03)	0.82 (0.03)	0.98 (0.16)	1.15 (1.17)
T₄	14.65	16.53 (1.88)	18.80 (2.27)	17.08 (1.72)	15.46 (1.62)	71.85	62.20 (9.65)	70.26 (8.06)	77.87 (7.61)	80.57 (2.70)	0.68	0.72 (0.04)	0.76 (0.04)	0.59 (0.17)	0.46 (0.13)
T₅	7.00	10.55 (3.55)	8.26 (2.29)	7.61 (0.65)	13.59 (5.98)	80.20	84.20 (4.00)	82.02 (2.18)	82.60 (0.58)	83.10 (0.50)	0.70	0.85 (0.15)	0.80 (0.05)	0.82 (0.02)	0.83 (0.01)
T₆	9.30	7.71 (1.59)	7.51 (0.20)	7.24 (0.27)	10.43 (3.19)	79.50	77.61 (1.89)	75.52 (2.09)	78.30 (2.78)	80.61 (2.31)	0.60	0.62 (0.02)	0.71 (0.09)	0.76 (0.05)	0.78 (0.02)
C.D	1.033	1.172	1.294	1.104	0.968	5.463	5.386	5.420	5.425	5.522	0.028	0.057	0.059	0.055	0.059
SE (m)	0.332	0.376	0.415	0.354	0.311	1.753	1.729	1.740	1.741	1.772	0.009	0.018	0.019	0.018	0.019
C.V	4.068	4.066	4.203	4.162	3.950	3.938	3.949	3.936	3.953	3.961	2.071	3.896	3.966	3.803	4.166

T₁-M₁-C₁- Composting without pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Fig 4.1 Contents of Total solids (%), total volatile solids (%) and volatile fatty acids in agricultural waste with pretreatment at different intervals.

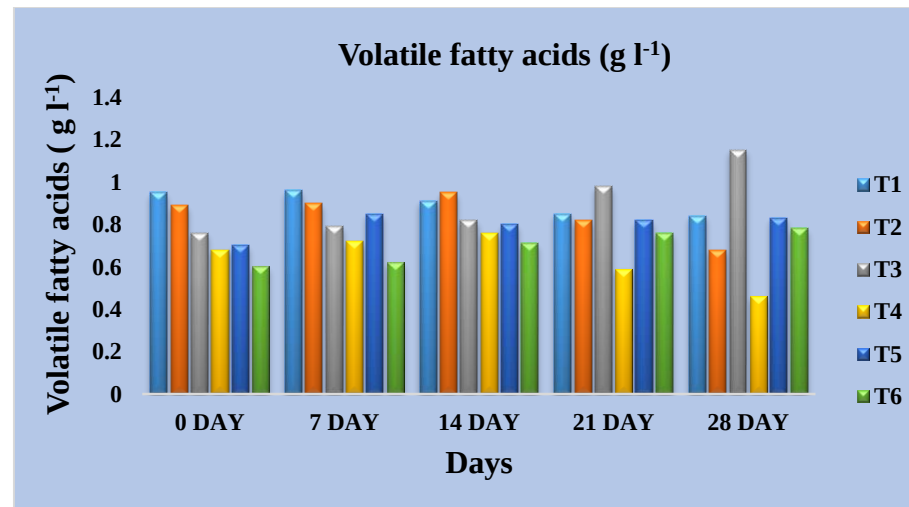
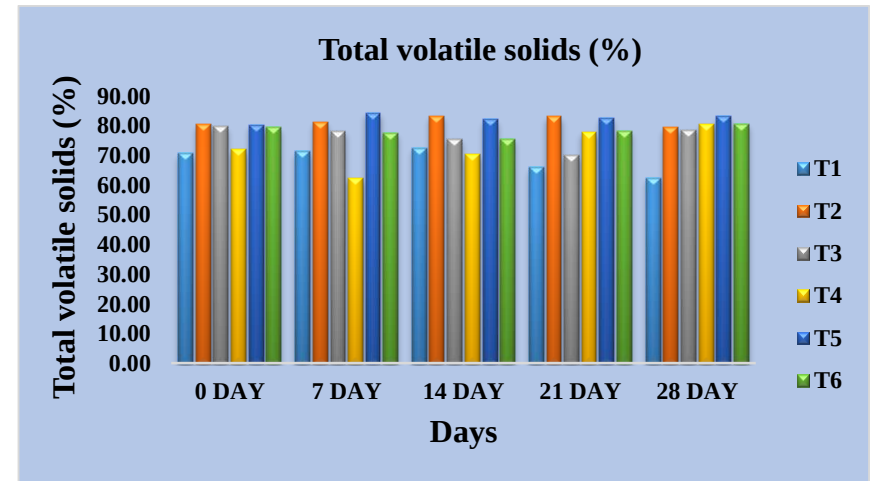
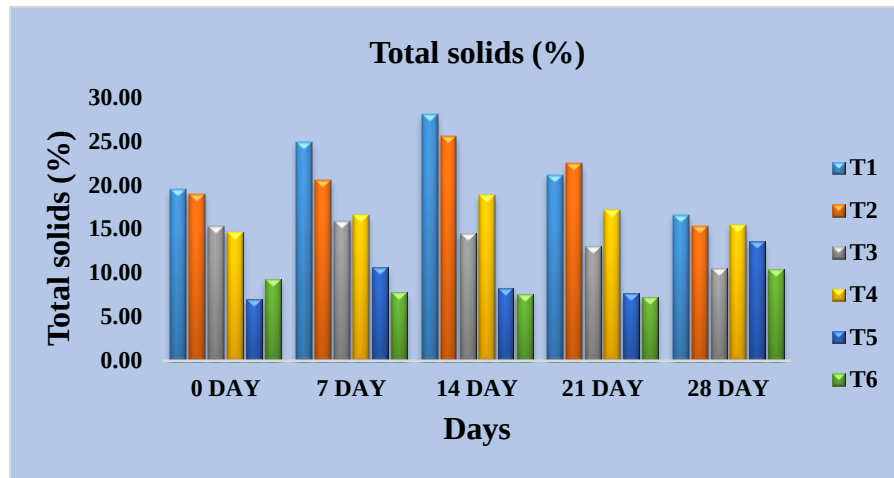


Table 4.5. pH in different treatments with pretreatment of agriculture waste

pH	0 Day (%)	7 Day (%)	14 Day (%)	21 Day (%)	28 Day (%)
T₁	8.00	8.14 (0.14)	8.25 (0.11)	8.38 (0.13)	8.18 (0.20)
T₂	8.20	8.25 (0.05)	8.38 (0.13)	8.40 (0.02)	8.26 (0.14)
T₃	8.25	8.37 (0.12)	8.40 (0.03)	8.26 (0.14)	8.33 (0.07)
T₄	8.20	8.24 (0.04)	8.36 (0.12)	8.27 (0.09)	8.28 (0.01)
T₅	6.20	7.11 (0.91)	6.92 (0.19)	6.72 (0.20)	6.38 (0.34)
T₆	6.30	6.56 (0.26)	7.12 (0.56)	6.49 (0.63)	6.61 (0.12)
C.D	0.535	0.421	0.287	0.550	0.543
SE (m)	0.172	0.135	0.092	0.177	0.174
C.V	3.950	3.011	2.021	3.943	3.931

AW- Agricultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

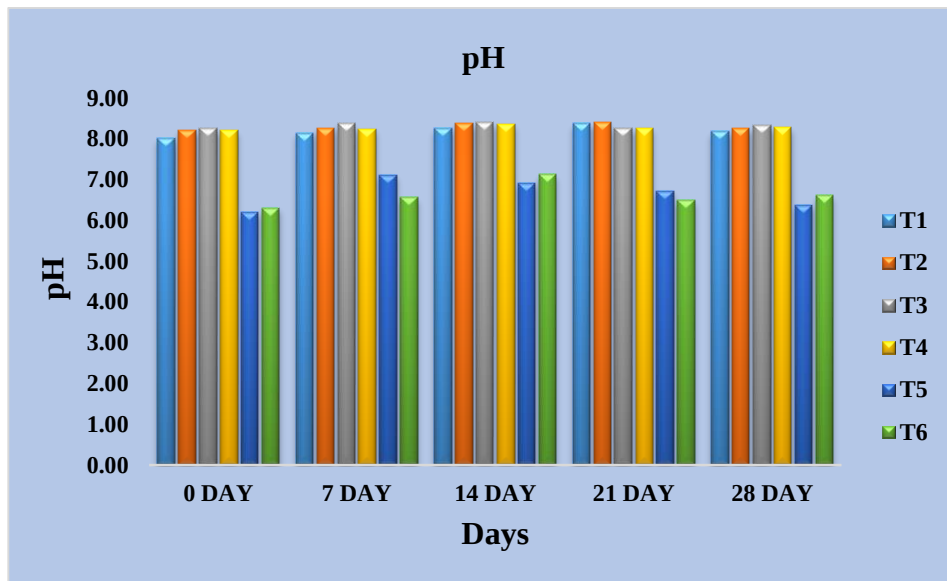
T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

*The table values with in the brackets indicate the difference between the values of adjacent days

Fig 4.2 pH in different treatments with pretreatment of agriculture waste



AW- Agricultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

was observed. Among all treatments T₃ (Vermicomposting without pretreatment) recorded the highest i.e. maximum pH (i.e., 8.25 to 8.40) followed by T₂ (Composting with pretreatment) (8.20 to 8.40) and T₄ (Vermicomposting with pretreatment) (8.20 to 8.36) and least pH was observed with the treatments T₅ (Biogas production without pretreatment) (i.e., 6.20 to 7.11) (Table 4.5). The pH of both vermicompost and compost was found to be in the range of 8.25-8.00 initially and dropped to 8.14 during the process. The slight change in pH from slightly acidic to neutral is due to increase in N, P and K content or Organic matter content. The results found that pH of the substrate has a significant effect on composting, vermicomposting and biogas production, because it affects the activity of bacteria to degrade organic matter into biogas. A low pH in the digester inhibits the activity of microorganisms involved in the digestion process particularly methanogenic bacteria.

In the above result pH in the vermicompost was 8.25 to 8.33 these results were higher when compared to the studies by Tognetti *et al.* (2005) who did work on composting vs. vermicomposting: a comparison of end product quality and recorded the pH was 7.7.

4.2.2.3 Contents of Nitrogen, Phosphorus and Potassium

Nitrogen content during pretreatment of agricultural waste found on par at different intervals in all the treatments except T₃ (Vermicomposting without pretreatment) (i.e. 0.84 to 1.65 %). Among all the intervals the N content gradually increased from 0th day to 28th day. Among the treatments the N content was found significantly highest with the treatment T₄ (Vermicomposting with pretreatment) (i.e. 1.81 to 2.41 %) on 28th day followed by T₃ (Vermicomposting without pretreatment) T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment). Lowest N content was observed in T₁ (Composting without pretreatment) (Table 4.6). There was a significant variation in available nitrogen content in substrates between different treatments. This variation in available nitrogen content of substrates was noticed in all the stages of composting, vermicomposting and biogas production period.

P content was found on par at different intervals in all the treatments. Among all the treatments P content was found significantly highest in T₅ (Biogas production without pretreatment) (i.e. 1.50 to 1.85 %) on 0th day followed by T₆ (Biogas production with pretreatment), T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment) and least was found in the treatment T₁ (Composting without

pretreatment). Among all the intervals P content was increased up to the 14th day of pretreatment and gradual reduction was observed up to 28th day (Table 4.6). It is evident from this experiment that increase in phosphatase activity by microorganisms leads to increase in amount of phosphorus which support the phosphate availability in the substrates.

Similarly the available potassium was on par from treatments of T₄ (Composting without pretreatment) to T₆ (Vermicomposting without pretreatment) on 0th day (i.e. 1.00 to 1.00 %) And among all the treatments T₄ (Vermicomposting with pretreatment) (i.e. 1.00 to 1.26 %) showed the highest K content on 28th day followed by T₆ (Vermicomposting with pretreatment) and least was observed with treatment T₁ (Composting without pretreatment) on 28th day. Among all the intervals the K content gradually increased from 0th day to 28th day in T₂ (Composting with pretreatment) (Table 4.6). This could be attributed to the fact that with the passage of time the substrate composition changes and becomes suitable for microorganisms to work upon, in turn increasing the activity of potassium in substrates between different intervals.

The breakdown of organic matter during the composting process is dependent on several factors working in concert. These include moisture, microbial populations, Oxygen (O₂), and a balance of Carbon (C) and Nitrogen (N). Microorganisms in the organic matter (OM) consume the readily available carbon. As it is metabolized, temperatures increase in the compost pile and Carbon dioxide (CO₂) is released. As a result, the pile is newly populated with thermophilic, or heat-loving, bacteria that consume the rest of the degradable carbon. As microbial activity slows, temperature decreases, allowing for colonization by fungi that slowly consume much of the remaining recalcitrant forms of lignins and cellulose. The resulting crumbly, earthy humus is considerably more stable than manure, meaning that its nutrients are less likely to be lost to leaching or volatilization into the atmosphere. Nitrogen losses impact negatively on the manure composting process, by decreasing nutrient concentration and hence compost quality, and generate health and environmental problems. Nitrogen losses through composting can occur by NH₃-volatilisation, leaching and denitrification. Denitrification can occur as a result of the development of anaerobic microsites within the material. Thus, the aerobic conditions of the compost should be ensured throughout the process, indicated that emission rates of N₂O–N were very much lower (about 10 times) than those of NH₃–N during composting of cattle manure with maize straw. Six treatments had more N, P and K % in different intervals as compared to the other treatments and this results were

similar to the work of Barik *et al.* (2011) who reported N (0.51 and 1.61 %), P (0.19 and 1.02 %) and K (0.15 and 0.73 %) in the vermicompost from agricultural wastes.

Table 4.6. Nitrogen, Phosphorus and Potassium content in different treatments with pretreatment of agricultural waste at different intervals

Agricultural waste	N (%)					P (%)					K (%)				
	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day
T₁	0.60	0.61 (0.01)	0.65 (0.04)	0.68 (0.03)	0.70 (0.02)	0.15	0.20 (0.05)	0.24 (0.04)	0.19 (0.05)	0.17 (0.02)	0.65	0.67 (0.02)	0.71 (0.04)	0.74 (0.03)	0.72 (0.02)
T₂	0.70	0.74 (0.04)	0.79 (0.05)	0.86 (0.07)	0.90 (0.04)	0.16	0.18 (0.02)	0.25 (0.07)	0.20 (0.05)	0.19 (0.01)	0.70	0.72 (0.02)	0.76 (0.04)	0.77 (0.01)	0.78 (0.01)
T₃	1.65	1.13 (0.52)	0.84 (0.29)	0.99 (0.15)	1.91 (0.92)	1.00	1.23 (0.23)	1.28 (0.05)	1.19 (0.09)	1.06 (0.13)	0.99	1.02 (0.03)	1.15 (0.13)	1.10 (0.05)	1.01 (0.09)
T₄	1.84	1.81 (0.03)	1.86 (0.05)	1.99 (0.13)	2.41 (0.42)	1.00	1.21 (0.21)	1.26 (0.05)	1.14 (0.12)	1.10 (0.04)	1.00	1.13 (0.13)	1.26 (0.13)	1.15 (0.11)	1.05 (0.10)
T₅	1.68	1.70 (0.02)	1.72 (0.02)	1.77 (0.05)	1.80 (0.03)	1.50	1.58 (0.08)	1.66 (0.08)	1.80 (0.14)	1.85 (0.05)	1.09	0.81 (0.28)	0.85 (0.04)	1.03 (0.18)	1.24 (0.21)
T₆	1.50	1.53 (0.03)	1.58 (0.05)	1.61 (0.03)	1.69 (0.08)	1.30	1.33 (0.03)	1.35 (0.02)	1.40 (0.05)	1.43 (0.03)	1.00	0.84 (0.16)	0.80 (0.04)	0.98 (0.18)	1.11 (0.13)
C.D	0.080	0.070	0.048	0.099	0.124	0.071	0.064	0.044	0.086	0.062	0.037	0.046	0.037	0.069	0.034
SE(m)	0.030	0.023	0.015	0.032	0.040	0.023	0.020	0.014	0.028	0.020	0.012	0.015	0.012	0.022	0.011
C.V	3.290	3.124	2.159	4.162	4.388	4.653	3.702	2.443	4.843	3.584	2.444	2.947	2.259	3.988	1.899

T₁-M₁-C₁- Composting without pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

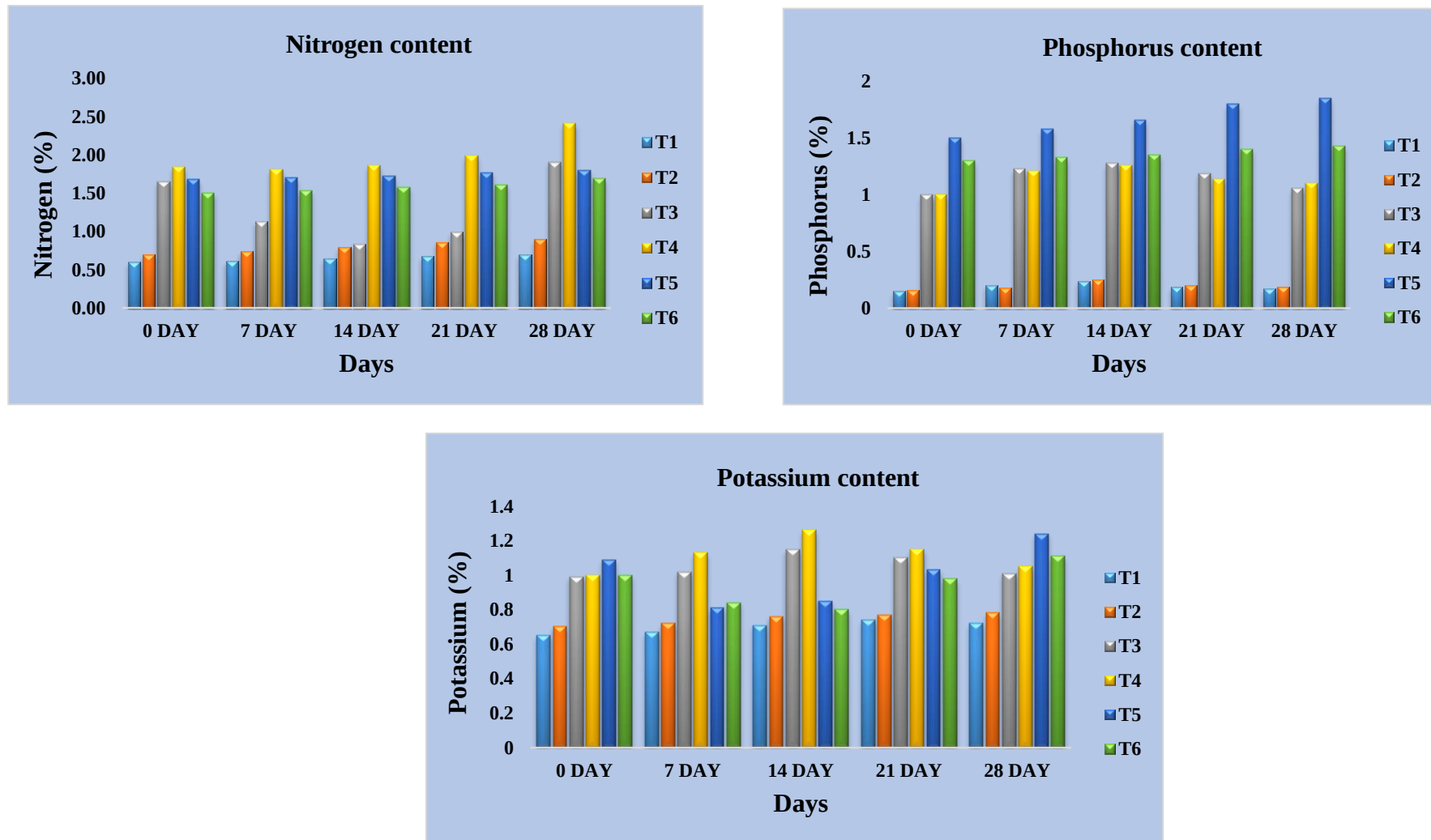
T₂-M₁-C₂- Composting with pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Fig 4.3 Nitrogen, Phosphorus and Potassium content in different treatments with pretreatment of agricultural waste at different intervals.



4.2.2.4 Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

Available organic carbon per cent in the pretreated agricultural waste on par among the treatments of T₁ (Composting without pretreatment) and T₂ (Composting without pretreatment) (i.e. 25.50 and 24.00) on 0th day and 28th day. The organic carbon content was found initially low and increased up to 14th day among the treatments. The organic carbon content was significantly highest with the treatment T₆ (Biogas production with pretreatment) (i.e., 45.98 to 46.86 %) followed by T₅ (Biogas production without pretreatment) (i.e., 33.40 to 35.20 %) and the least was found with treatment T₃ (Vermicomposting without pretreatment) (i.e., 21.60 to 25.00 %) on 0th day to 28th day (Table 4.7). Micro-organisms require C, N, Phosphorus (P) and Potassium (K) as the primary nutrients of particular importance in the C: N ratio of raw materials. The optimal C: N ratio of raw materials is between 25:1 and 30:1 although ratios between 20:1 and 40:1 are also acceptable. Where the ratio is higher than 40:1, the growth of micro-organisms is limited, resulting in a longer composting time. C: N ratio of less than 20:1 leads to under utilization of N and the excess may be lost to the atmosphere as ammonia or nitrous oxide, and odour can be a problem.

The biological oxygen demand in the pretreated agricultural waste was significantly increased. Among the different treatments the results were found on par with the treatments T₃ (Vermicomposting without pretreatment), T₅ (Biogas production without pretreatment) on 0th day and T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment) on 28th day. The BOD value was low initially at the 0th day and increased up to 28th day among the treatments except T₄ (Vermicomposting with pretreatment) and T₅ (Biogas production without pretreatment). The BOD value was highest with the treatment T₆ (Biogas production with pretreatment) (i.e. 106.80 mg l⁻¹) and found lowest with the treatment T₁ (Composting without pretreatment) (Table 4.7). The biological oxygen demand was recorded more in all the treatments at different intervals. Hence, dissolved oxygen depletion is most likely to become evident during the initial aerobic composting and anaerobic digestion, microbial population increase in response to a large amount of organic material. If the microbial population deoxygenates the water, however, that lack of oxygen imposes a limit on population growth of microorganisms.

The concentration of COD among all the treatments was found on par with the treatments T₁ (Composting without pretreatment) and T₂ (Composting with pretreatment)

Table 4.7. Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments as pretreatment of agricultural waste at different intervals.

Agricultural waste	OC (%)					BOD (mg l ⁻¹)					COD (g l ⁻¹)				
	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day
T₁	25.50	28.00 (2.50)	28.15 (0.15)	28.30 (0.15)	26.70 (1.60)	92.00	94.00 (2.00)	93.60 (0.40)	94.70 (1.10)	95.90 (1.20)	98.80	97.52 (1.28)	99.50 (1.98)	99.62 (0.12)	101.30 (1.68)
T₂	24.00	25.40 (1.40)	26.78 (1.38)	26.13 (0.65)	25.00 (1.13)	93.50	95.20 (1.70)	95.60 (0.40)	96.70 (1.10)	97.20 (0.50)	95.00	96.58 (1.58)	98.30 (1.72)	98.40 (0.10)	99.20 (0.80)
T₃	21.60	22.05 (0.45)	24.50 (2.45)	25.00 (0.50)	22.00 (3.00)	95.40	96.03 (0.63)	97.30 (1.27)	98.20 (0.90)	99.10 (0.90)	110.20	110.45 (0.25)	100.78 (9.67)	101.45 (0.67)	98.79 (2.66)
T₄	22.60	24.45 (1.85)	26.48 (2.03)	22.57 (3.91)	23.50 (0.93)	98.30	97.01 (1.29)	98.40 (1.39)	99.00 (0.60)	98.20 (0.80)	120.40	118.30 (2.10)	104.86 (13.44)	106.89 (2.03)	97.23 (9.66)
T₅	33.40	35.12 (1.72)	34.18 (0.94)	33.58 (1.40)	35.20 (1.62)	95.20	94.60 (0.60)	99.12 (4.52)	98.20 (0.92)	101.20 (3.00)	100.30	99.08 (1.22)	99.42 (0.34)	99.85 (0.43)	110.30 (10.45)
T₆	45.60	45.66 (0.06)	46.86 (1.20)	45.98 (0.88)	46.00 (0.02)	96.75	96.80 (0.05)	99.14 (2.34)	99.80 (0.66)	106.80 (7.00)	101.60	100.45 (1.15)	100.98 (0.53)	98.56 (2.42)	109.60 (11.04)
C.D	2.125	1.680	1.155	2.213	2.192	1.409	1.406	1.439	1.438	1.470	3.769	5.613	3.625	5.443	5.553
SE(m)	0.682	0.539	0.371	0.710	0.704	0.452	0.451	0.462	0.461	0.472	1.210	1.802	1.164	1.747	1.782
C.V	4.103	3.101	2.060	4.063	4.097	0.824	0.819	0.824	0.819	0.821	2.007	3.008	2.003	3.002	3.005

T₁-M₁-C₁- Composting without pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

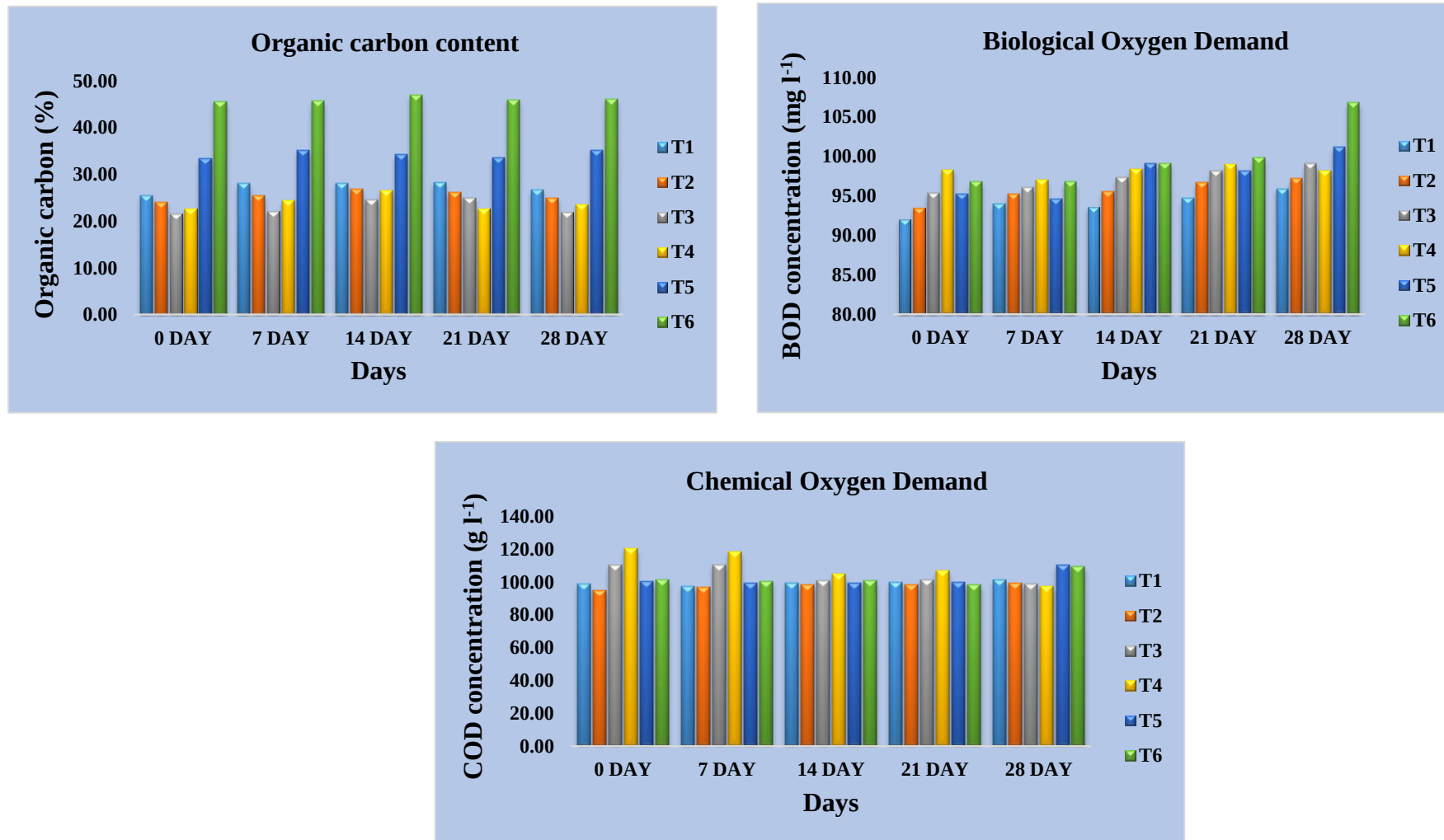
T₂-M₁-C₂- Composting with pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Fig 4.4 Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments as pretreatment of agricultural waste at different intervals.



(i.e. 95.00 and 98.80 g l⁻¹), T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) on 0th day (100.30 and 101.60 g l⁻¹). COD value was found highest with the treatment T₄ (Vermicomposting with pretreatment) (120.40 g l⁻¹) followed by T₃ (Vermicomposting without pretreatment) and found lowest with the treatment T₂ (Composting with pretreatment) (95.00 g l⁻¹) on 0th day. All the treatments were found to be on par with the treatments T₅ (Biogas production without pretreatment) (110.30 to 109.60 g l⁻¹) and T₁ (Composting without pretreatment) (101.30 to 97.23 g l⁻¹) on 28th day (Table 4.7). The limitations of this method includes pH reduction, the ammonia value increase with temperature increase, energy supply expenses, high values of ammonium, electrical conductivity and alkalinity can increase the toxicity in waste water, and this will effect BOD and COD values and biodegradation capability.

4.2.2.5 Cellulose content

The results of cellulose % in the pretreated agricultural waste revealed that, the cellulose content was highest observed in T₂ (Composting with pretreatment) (i.e. 27.58 %) on 0th day and increased upto 14th day. Lowest value of cellulose content was observed in T₁ (Composting without pretreatment) on 0th day and T₆ (Biogas production with pretreatment) on 28th day. On par results were found with all the treatments on 0th day. On par results were also observed in T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment), T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) on 28th day. (Table 4.8).

Populations growing in compost piles consist mainly of bacteria (including actinobacteria) and fungi. Polymers such as hemicellulose, cellulose, and lignin are only degraded once the more easily degradable compounds have been consumed. Afterwards, the lignocellulosic materials are partly transformed into humus. At the beginning of the composting mesophilic bacteria are predominant. Once the temperature has reached more than 40 °C, thermophilic bacteria and fungi appear in the compost piles. When the temperatures reaches more than 60 °C, the microbial activity decrease, and as the compost pile cools, mesophilic bacteria appear again. Except for 1 % of anaerobic bacteria in the compost process, which carry out much of the cellulolytic activity and so play a major role in the biodegradation of lignocellulosic materials, most biodegradation is carried out under aerobic conditions. The similar results were presented earlier by Mohan *et al.* (2012) where they have recorded more cellulose content in the pretreated rice straw than untreated rice straw. The biological pretreatment of rice straw was done using microorganisms.

Table 4.8. Cellulose content in different treatments as pretreatment of agriculture waste

Agricultural waste	0 Day (%)	7 Day (%)	14 Day (%)	21 Day (%)	28 Day (%)
T₁	24.31	24.44 (0.13)	24.89 (0.45)	23.27 (1.62)	23.02 (0.25)
T₂	27.58	27.85 (0.27)	27.99 (0.14)	26.02 (1.97)	22.98 (3.04)
T₃	24.79	25.65 (0.86)	24.53 (1.12)	22.07 (2.46)	22.05 (0.02)
T₄	26.10	27.46 (1.36)	26.95 (0.51)	20.53 (6.42)	21.10 (0.57)
T₅	25.33	24.75 (0.58)	22.16 (2.59)	17.33 (4.83)	16.00 (1.33)
T₆	25.89	26.18 (0.29)	23.35 (2.83)	17.03 (6.32)	15.85 (1.18)
C.D	1.814	1.427	1.369	1.149	1.429
SE(m)	0.582	0.458	0.440	0.369	0.459
C.V	3.927	3.044	3.047	3.036	3.936

AW- Agricultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

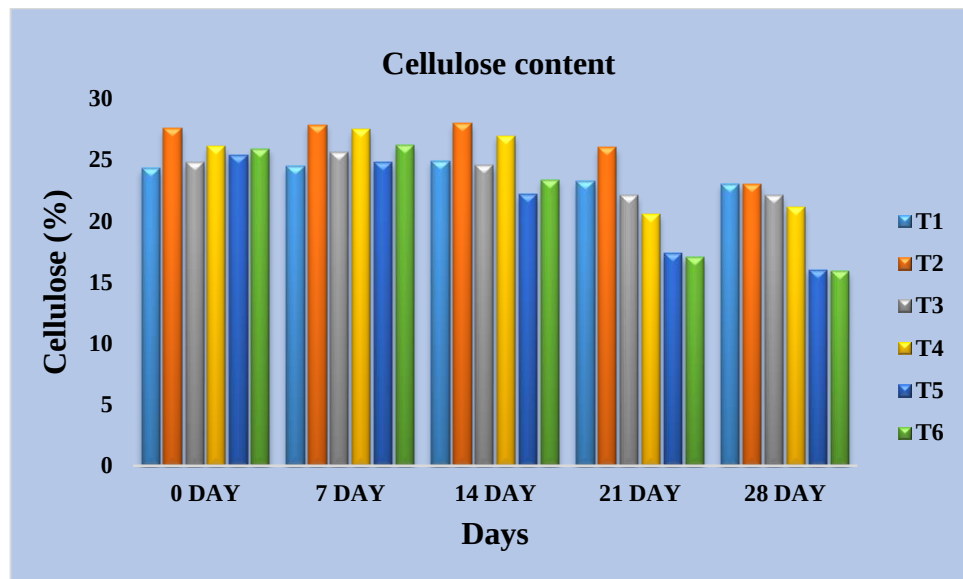
T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

*The table values with in the brackets indicate the difference between the values of adjacent days

Fig 4.5 Cellulose content in different treatments with pretreatment of agriculture waste



AW- Agricultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

4.2.2.6 Population of bacteria, fungi and actinomycetes

Among the treatments the bacterial population count was found significantly highest with the treatment T₁ (Composting without pretreatment) (i.e. 9.9×10^6 CFU g⁻¹) followed by T₂ (Composting with pretreatment) and least was observed with the treatment T₆ (Biogas production with pretreatment) (3.2×10^6 CFU g⁻¹). Fungal population counts was varied from 2.3 to 4.4×10^4 CFU g⁻¹ and 2.7 to 4.5×10^4 CFU g⁻¹ on 0th and 28th day respectively. Among the treatments fungal population was found significantly highest with the treatment T₆ (Biogas production with pretreatment) (4.8×10^4 CFU g⁻¹) on 21st day followed by T₅ (Biogas production without pretreatment) and lowest fungal population was found with T₁ (Composting without pretreatment) (2.3×10^4 CFU g⁻¹). Among the intervals the actinomycetes population gradually increased with the increase in the no. of days up to 21st day decreased in the population was observed on 28th day. Among the treatments the population was found highest with the treatment T₁ (Composting without pretreatment) (9.8×10^4 CFU g⁻¹) on 0th day followed by T₂ (Composting with pretreatment) and least population was found with the treatment T₅ (Biogas production without pretreatment) (4.2×10^4 CFU g⁻¹) (Table 4.9). The increase in temperature during the composting process was caused by the heat generation from the respiration and decomposition of sugar, starch and protein by the population of microorganisms. The increment in temperature is a good indicator that there is microbial activity in the compost pile, as a higher temperature denotes greater microbial activity. Continued metabolism depends on sufficient aeration. Average O₂ concentration inside the compost mix is 15 to 20 %, while CO₂ is 0.5 to 5 %. When O₂ content falls below these levels, anaerobic microbial populations surpass aerobic species. As a result, malodorous fatty acids and methane levels may increase. Because oxygen consumption is a function of microbial activity, oxygen levels are also related to substrate temperatures. Temperatures between 28 and 55 °C are optimal, few species of bacteria can survive at temperatures above 70 °C. Fungi capable of degrading cellulose and lignin are also greatly inhibited by high temperatures.

Total microbial population in the agriculture waste was more in the results of Barakah *et al.* (2013) who demonstrated total bacterial counts at 3 week (32×10^4 CFU g⁻¹), at 6th week (54×10^4 CFU g⁻¹) and 9th week (63×10^4 CFU g⁻¹).

Table 4.9 Population of bacteria, fungi and actinomycetes in different pretreated substrates of agricultural waste

Agricultural waste	0 Day			7 Day			14 Day			21 Day			28 Day		
	B	F	A	B	F	A	B	F	A	B	F	A	B	F	A
T₁	9.90	2.30	9.80	9.50 (0.40)	2.30 (0.00)	9.70 (0.10)	9.00 (0.50)	2.40 (0.10)	9.50 (0.20)	8.80 (0.20)	2.50 (0.10)	9.20 (0.30)	8.00 (0.80)	2.70 (0.20)	8.00 (1.20)
T₂	6.90	3.40	8.20	6.50 (0.40)	3.50 (0.10)	8.30 (0.10)	6.40 (0.10)	3.70 (0.20)	8.40 (0.10)	6.30 (0.10)	3.90 (0.20)	8.60 (0.20)	6.00 (0.30)	3.90 (0.00)	6.50 (2.10)
T₃	4.50	3.60	7.20	4.40 (0.10)	3.70 (0.10)	7.30 (0.10)	4.30 (0.10)	3.80 (0.10)	7.40 (0.10)	4.20 (0.10)	4.00 (0.20)	7.60 (0.20)	4.00 (0.20)	4.10 (0.10)	5.00 (2.60)
T₄	4.80	3.30	6.50	4.70 (0.10)	3.40 (0.10)	6.60 (0.10)	4.60 (0.10)	3.60 (0.20)	6.40 (0.20)	4.50 (0.10)	3.80 (0.20)	6.10 (0.30)	4.10 (0.40)	3.80 (0.00)	5.90 (0.20)
T₅	4.60	4.20	4.20	4.50 (0.10)	4.30 (0.10)	4.30 (0.10)	4.40 (0.10)	4.50 (0.20)	4.50 (0.20)	4.30 (0.10)	4.40 (0.10)	4.70 (0.20)	4.00 (0.30)	3.90 (0.50)	3.60 (1.10)
T₆	3.20	4.40	4.60	3.30 (0.10)	4.50 (0.10)	4.70 (0.10)	3.40 (0.10)	4.70 (0.20)	4.90 (0.20)	3.60 (0.20)	4.80 (0.10)	4.10 (0.80)	3.80 (0.20)	4.50 (0.30)	3.90 (0.20)
C.D	0.430	0.180	0.254	0.315	0.199	0.383	0.305	0.206	0.381	0.301	0.212	0.378	0.280	0.208	0.309
SE(m)	0.138	0.058	0.082	0.101	0.064	0.123	0.098	0.066	0.122	0.097	0.068	0.121	0.090	0.067	0.099
C.V	4.230	2.830	2.095	3.193	3.052	3.121	3.168	3.033	3.090	3.165	3.016	3.131	3.123	3.024	3.132

AW- Agricultural waste (B: Bacteria (10^6 CFU g⁻¹); F: Fungi (10^4 CFU g⁻¹); A: Actinomycetes (10^4 CFU g⁻¹))

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

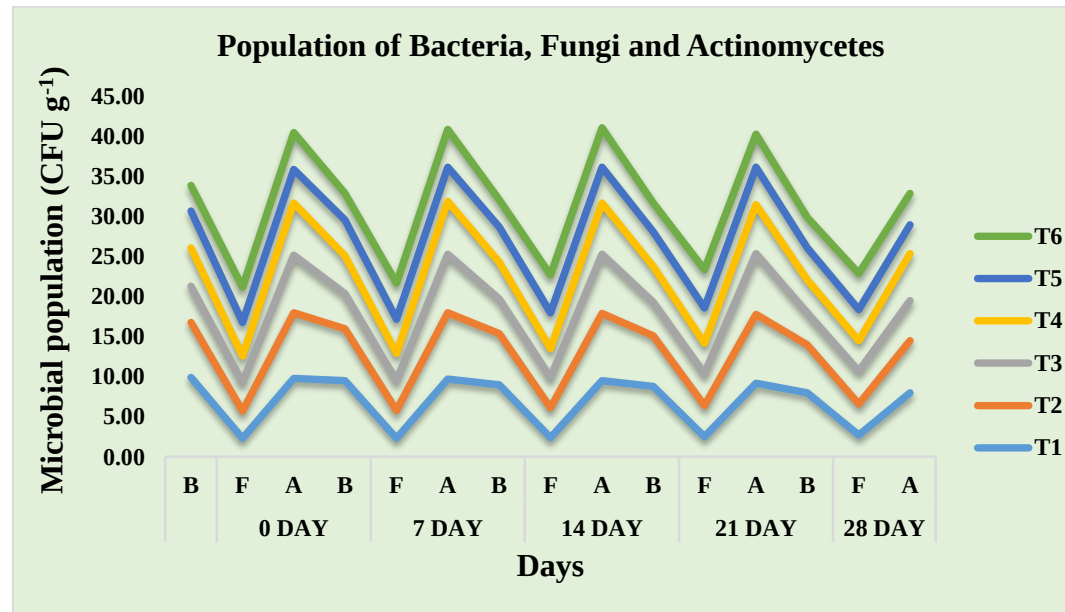
T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Fig 4.6 Population of bacteria, fungi and actinomycetes in different pretreated substrates of agricultural waste



AW- Agricultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

4.2.3 Aerobic Composting, Vermicomposting and Anaerobic Digestion of Agricultural Waste Enriched With and Without Cellulose Degrading Bacteria

4.2.3.1 Contents of Total Solids (TS %), Total Volatile Solids (TVS %) and Volatile Fatty Acids (VFA)

Total solids (%) in aerobic and anaerobic digestion of agricultural wastes among all the treatments was found to be on par in the treatments T₂ (Composting with pretreatment) (15.24 %), T₄ (Vermicomposting with pretreatment) (15.46 %) and T₃ (Vermicomposting without pretreatment), (10.45 %) T₆ (Biogas production with pretreatment) (10.43 %) on 0th day. Among all the treatments T₁ (Composting without pretreatment) (16.56 %) showed the highest significant result followed by T₄ (Vermicomposting with pretreatment) and lowest was observed with the treatment T₃ (Vermicomposting without pretreatment) (9.65 %). Among the intervals the TS % was found to decrease from 0th day to 60th day in all the treatments (Table 4.10).

Total solids per cent in the agriculture waste was similar (i.e. 7.05 % and 8.76 % at 60th day in the treatments T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) to the results of Yavini *et al.* (2014) who demonstrated that agriculture waste (maize and rice) were anaerobically digested in a laboratory scale reactor at mesophilic conditions (33 - 35 °C). The total solids per cent in the substrates were estimated as 7.0 % and 9.0 % in agriculture waste.

Total volatile solids % in the agricultural waste in different treatments was found to be on par in the treatments T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment) on 0th day, 15th day, 30th day, 45th day and 60th day. Among all the treatments TVS % was found highest in the treatment T₅ (Biogas production without pretreatment) followed by T₆ (Biogas production with pretreatment), T₄ (Vermicomposting with pretreatment) and least was observed in T₁ (Composting without pretreatment) on 0th day. Whereas T₃ (Vermicomposting without pretreatment) was found to be highest TVS % on 60th day which was found on par with the treatments T₂ (Composting with pretreatment) and T₄ (Vermicomposting with pretreatment) and least was found in the treatment T₅ (Biogas production without pretreatment) on 60th day. Among all the intervals TVS % gradually decreased with increase in the no. of days except in T₅ (Biogas production without pretreatment) (Table 4.10). Most of the biogas sludge contains the Total volatile solids (TVS %) was 83.04 to 85.14 % is optimum for the best biogas production under suitable conditions.

Total volatile solids per cent in the agriculture waste was similar to the results of Yangyang *et al.* (2016) who demonstrated that anaerobic digestion of corn stover in a laboratory scale reactor at mesophilic conditions (35 °C). The total volatile solids % in the substrates were estimated as 76.3 and 87.5 % in agriculture waste (corn stover digestion) respectively.

Total volatile solids % in the horticulture waste was similar to the results of Velmurugan *et al.* (2011) demonstrated that the anaerobic digestion of vegetable wastes for biogas production in a fed-batch reactor and recorded total volatile solids % in the substrates was 83.10, 86.43 % in fruit waste and 80.40 % in vegetable waste.

The treatments T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) had more total solids (83.10 % and 80.61 %) as compared to the other treatments and these results were similar to the results observed by Ofoefule *et al.* (2010) who reported TVS % in biogas slurry (36.38 % in cow dung and 27.01 % in swine dung).

Among all the treatments the lowest VFA % was found in the treatment T₄ (Vermicomposting with pretreatment) (0.46 g l⁻¹) and highest was found with T₃ (Vermicomposting without pretreatment) (1.15 g l⁻¹) on 0th day. During 60th day highest VFA was found with T₆ (Biogas production with pretreatment) (2.26 g l⁻¹) and lowest was observed with T₄ (Vermicomposting with pretreatment) (0.38 g l⁻¹). Among all the intervals, first four treatments showed gradual decrease in VFA % from 0th day to 60th day which was found vice versa with the treatments T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) (Table 4.10). Most of the biogas sludge contains the Volatile Fatty Acids (VFA) content was 0.28-0.30 g l⁻¹ is optimum for the best biogas production under suitable conditions.

Volatile fatty acids % in the agriculture waste was similar to the results of Yangyang *et al.* (2016) who demonstrated that anaerobic digestion of corn stover in a laboratory scale reactor at mesophilic conditions (35 °C) and recorded volatile fatty acids % in the substrates were estimated as 0.64 % in agriculture waste (corn stover digestion). Total VFA, acetate and propionate increased immediately as a

Table 4.10 Total solids (%), total volatile solids (%) and volatile fatty acids content in aerobic composting and anaerobic digestion of agricultural waste at different intervals.

Agricultural waste	TS (%)					TVS (%)					VFA (g l ⁻¹)				
	0 Day	15 Day	30 Day	45 Day	60 Day	0 Day	15 Day	30 Day	45 Day	60 Day	0 Day	15 Day	30 Day	45 Day	60 Day
T₁	16.56	16.02 (0.54)	15.88 (0.14)	15.30 (0.58)	14.99 (0.31)	62.38	61.78 (0.60)	60.25 (1.53)	59.78 (0.47)	58.02 (1.76)	0.84	0.81 (0.03)	0.79 (0.02)	0.76 (0.03)	0.72 (0.04)
T₂	15.24	15.13 (0.24)	15.00 (0.13)	14.78 (0.22)	14.03 (0.75)	79.52	79.45 (0.07)	78.12 (1.33)	76.68 (1.44)	76.08 (0.60)	0.68	0.65 (0.03)	0.60 (0.05)	0.58 (0.02)	0.55 (0.03)
T₃	10.45	10.38 (0.07)	10.05 (0.33)	9.65 (0.40)	9.54 (0.11)	78.56	78.46 (0.10)	77.12 (1.34)	77.32 (0.20)	76.48 (0.84)	1.15	1.00 (0.15)	0.99 (0.01)	0.97 (0.02)	0.94 (0.03)
T₄	15.46	15.30 (0.16)	15.07 (0.23)	14.88 (0.19)	14.15 (0.73)	80.57	79.58 (0.99)	78.62 (0.96)	76.85 (1.77)	75.65 (1.20)	0.46	0.45 (0.01)	0.43 (0.02)	0.40 (0.03)	0.38 (0.02)
T₅	13.59	12.45 (1.14)	10.10 (2.35)	11.40 (1.30)	7.05 (4.35)	83.10	81.20 (1.90)	52.94 (28.26)	68.29 (15.35)	45.29 (23.00)	0.83	0.65 (0.18)	1.72 (1.07)	2.12 (0.40)	1.85 (0.27)
T₆	10.43	11.36 (0.93)	12.52 (1.16)	11.20 (1.32)	8.76 (2.44)	80.61	82.56 (1.95)	77.16 (5.40)	69.14 (8.02)	64.47 (4.67)	0.78	0.62 (0.16)	1.59 (0.97)	2.09 (0.50)	2.26 (0.17)
C.D.	0.968	0.732	0.721	0.706	0.636	5.522	4.182	3.851	3.865	3.611	0.059	0.037	0.061	0.070	0.072
SE(m)	0.311	0.235	0.231	0.226	0.204	1.772	1.342	1.236	1.241	1.159	0.019	0.012	0.019	0.022	0.023
C.V.	3.950	3.027	3.057	3.048	3.098	3.961	3.012	3.028	3.012	3.042	4.166	2.988	3.301	3.358	3.605

T₁-M₁-C₁- Composting without pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

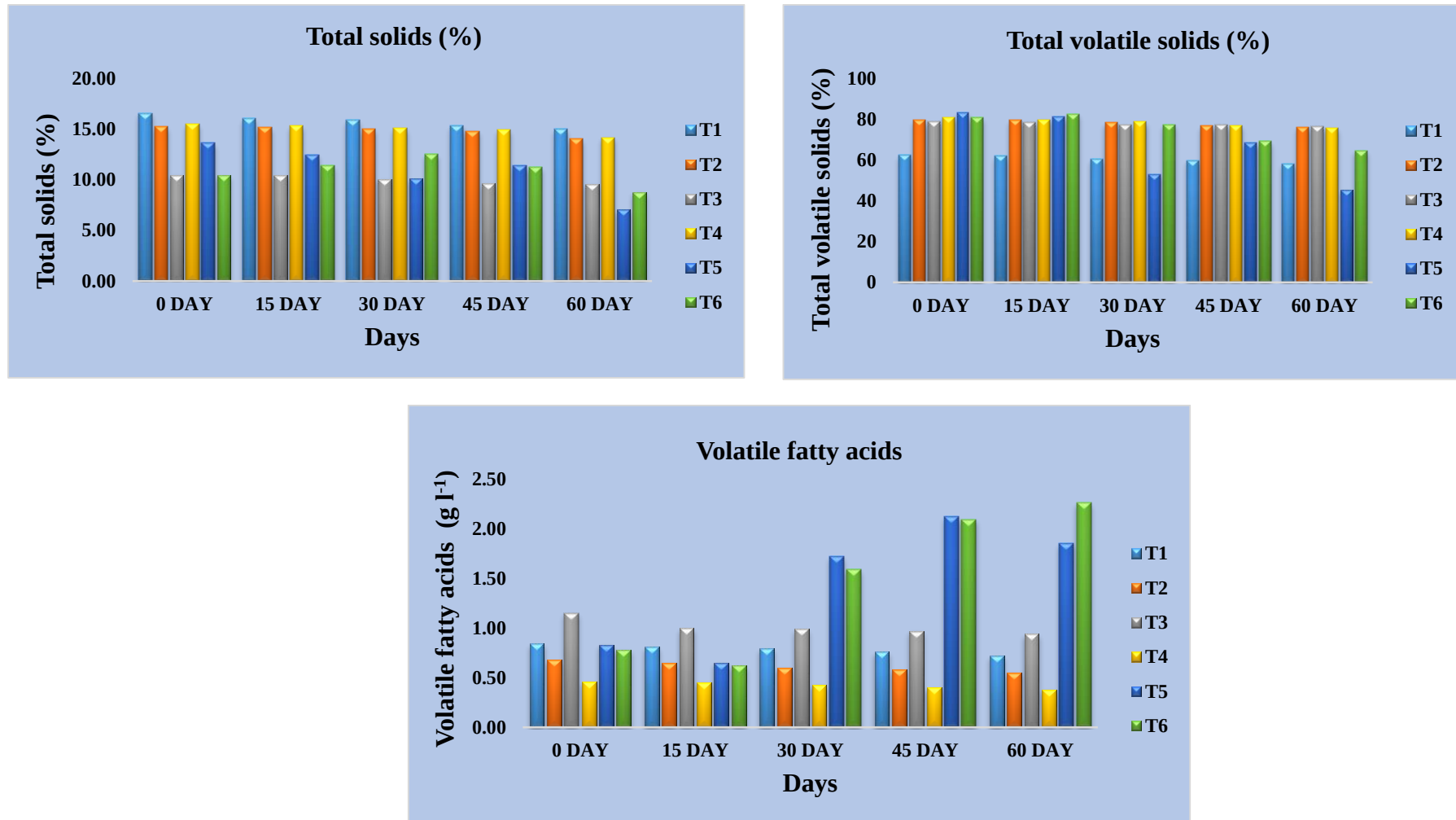
T₂-M₁-C₂- Composting with pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Fig 4.7 Total solids (%), total volatile solids (%) and volatile fatty acids in aerobic composting and anaerobic digestion of agricultural waste at different intervals.



result of sudden temperature decrease in the thermophilic reactors. VFA concentrations increased for more than 1 h, which was also partly due to the addition of acids in the feed which were not metabolized due to decrease of metabolic activity at the low temperature for 24 h. However, VFA variation was relatively stable in the low temperature duration. These results were presumably attributable to the temperature fluctuation which decreased the hydrolysis and anaerobic digestion activity. During acidogenesis, the products of hydrolysis are converted by acidogenic (fermentative) bacteria into methanogenic substrates. Simple sugars, amino acids and fatty acids are degraded into acetate, carbon dioxide and hydrogen (70 %) as well as into Volatile Fatty Acids (VFA) and alcohols (30 %).

4.2.3.2 pH

Slightly acidic pH values recorded in T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) treatment and alkaline pH was recorded in T₁ (Composting without pretreatment) to T₄ (Vermicomposting with pretreatment) initially. During 60th day all the treatments were recorded with near neutral pH (i.e. 8.18 to 8.33) (Table 4.11). Ideal pH for obtaining best compost was 8.0-8.5, and vermicompost is 6.9-7.0 this was varied from substrate to substrate and also different from the complete process of the composting period.

pH in the agriculture waste was similar to the results of Yangyang *et al.* (2016) who demonstrated that anaerobic digestion of corn stover in a laboratory scale reactor at mesophilic conditions (35 °C). The pH in the substrates were estimated as 7.4, 7.5, 7.7 and 8.1 in agriculture waste at different treatments (corn stover digestion).

The variation in pH between different treatments at different intervals of composting, vermicomposting and biogas production may be due to variation in chemical characteristics of substrates used and organisms involved in degradation as this was noticed in the study conducted by Chandrashekara *et al.* (2011) where, they have recorded more pH in the compost prepared by using earth worms (7.4) compared to compost prepared by without inoculums (6.5) and also with microbial consortium (6.8). pH recorded in the treatments of T₂ (Composting with

Table 4.11. pH in the slurry samples

AW	pH				
	0 Day	15 Day	30 Day	45 Day	60 Day
T₁	8.18	8.12 (0.06)	7.58 (0.54)	7.86 (0.28)	7.58 (0.28)
T₂	8.26	8.53 (0.27)	7.71 (0.82)	8.16 (0.45)	7.45 (0.71)
T₃	8.33	7.95 (0.38)	7.47 (0.48)	6.60 (0.87)	7.30 (0.70)
T₄	8.28	7.19 (1.09)	6.80 (0.39)	6.30 (0.50)	7.21 (0.91)
T₅	6.38	6.17 (0.21)	7.58 (1.41)	7.86 (0.28)	7.58 (0.28)
T₆	6.61	6.13 (0.48)	7.71 (1.58)	8.16 (0.45)	7.45 (0.71)
C.D.	0.233	0.313	0.323	0.384	0.396
SE(m)	0.075	0.100	0.104	0.123	0.128
C.V.	1.691	2.368	2.402	2.852	2.994

AW- Agricultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

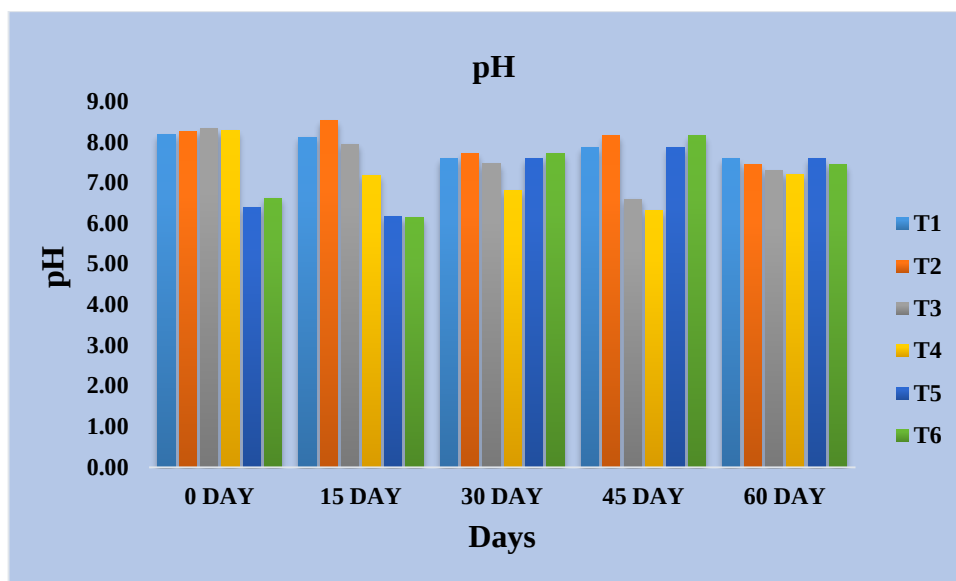
T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of threereplications

*The table values with in the brackets indicate the difference between the values of adjacent days

Fig: 4.8 pH in the slurry samples



T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

pretreatment), T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment) was similar as 7.45 and 6.80.

4.2.3.3 Contents of Nitrogen, Phosphorus and Potassium

N content during aerobic composting and anaerobic digestion of agricultural waste among all the treatments and different intervals was found on par in the treatments T₃ (Vermicomposting without pretreatment) and T₅ (Biogas production without pretreatment) on 0th day. Among all the treatments highest significant result was found in the treatment T₄ (Vermicomposting with pretreatment) (2.41 %) followed by T₃ (Vermicomposting without pretreatment) and least was found in T₁ (Composting without pretreatment) (0.70 %) on 0th day. Among all the intervals N content gradually increased in the treatments T₁ (Composting without pretreatment) and T₂ (Composting with pretreatment) from 0th day to 60th day (Table 4.12). The optimum N, P and K content in the compost (2.2, 0.85 and 0.90 %) and vermicompost (0.51-1.61, 0.7-0.9 and 0.15-0.73 %) under suitable conditions by using agricultural waste as a substrate.

The similar kind of results were noticed in the study conducted by Wani *et al.* (2013) where they have recorded more N % in cow dung (2.03 %) observed in the present experiment is similar to the results as 1.95 % (on 60th day in T₄) and 1.91 % (on 0th day in T₃) respectively.

The percentage of highest N content in T₄ (Vermicomposting with pretreatment) (2.41 and 1.80 %) and T₆ (1.69 %) from the present work as compared with the results presented by Jeyabal *et al.* (2001) who studied the composition of nutrients like N, P and K in biogas digested slurry and obtained the N (1.65 %).

The similar kind of results were noticed in the study conducted by Chandrashekara *et al.* (2011) where, they have recorded more nitrogen content in the compost prepared by using earth worms (1.90 %) compared to compost prepared by without inoculum (1.36 %) and also with microbial consortium (1.54 %).

Among all the treatments P content was found significantly highest in the treatment T₅ (Biogas production without pretreatment) (1.85 %) and least was found in T₁ (Composting without pretreatment). Among all the intervals increase in P content was found in treatments T₁ (Composting without pretreatment) and T₃ (Vermicomposting without pretreatment) and decrease in P content was observed in treatment T₅ (Biogas production without pretreatment) (Table 4.12). More increase in phosphorus in different

treatments is probably due to mineralization and mobilization of phosphorus due to microbial population. During organic matter decomposition by the microorganisms is the major mechanism for solubilisation of insoluble phosphorus, which subsequently results in increase in phosphorus content.

The percentage of highest P % in T₅ (Biogas production without pretreatment) (1.85, 1.77 and 1.65 %) and T₆ (Biogas production with pretreatment) (1.43, 1.37 and 1.65 %) from the present work as compared with the results presented by Jeyabal *et al.* (2001) who studied the composition of nutrients like N, P and K in biogas digested slurry recorded as P (0.70, 0.76 and 1.20 %).

The similar kind of results were noticed in the study conducted by Singh and Nain (2014) where, they have conducted an experiment microorganisms in the conversion of agricultural wastes to compost and recorded phosphorus content in the compost 0.8 % and in vermicompost 0.4 %.

The available potassium in the agricultural waste among all the treatments was found to be on par in T₁ (Composting without pretreatment), T₂ (Composting with pretreatment) (0.72 and 0.78 %) and T₃ (Vermicomposting without pretreatment), T₄ (Vermicomposting with pretreatment) (1.01 and 1.05 %). Among all the treatments K content was found to be highest in the treatment T₅ (Biogas production without pretreatment) (1.75 %) followed by T₃ (Vermicomposting without pretreatment) and least was found with the treatment T₁ (Composting without pretreatment) and among all the intervals K content was increased up to 45th day and decreased when it reached 60th day (Table 4.12).

The composting system and conditions, characteristics of both the bedding material and the bulking agent added for composting and even the environmental conditions of the season have a great influence on the mineralisation of the organic matter during composting. During the active phase of the composting process the organic carbon decreases in the material due to decomposition of the organic matter by the microorganisms. This loss of organic matter reduces the weight of the pile and decreases the C/N ratio. The degradation rate of the organic matter decreases gradually as composting progresses because of the reduction in available carbon sources, and synthesis reactions of new complex and polymerised organic compounds (humification) prevail over mineralisation during the maturation phase. In the results obtained as K per

cent in the T₅ (Biogas production without pretreatment) was more (1.36, 1.58, 1.75 and 1.42 %)

Plate: 4.4 Hot plate with samples kept for digestion



Table 4.12. Nitrogen, Phosphorus and Potassium content in different treatments during aerobic composting and anaerobic digestion of agricultural waste at different intervals.

Agricultural waste	N (%)					P (%)					K (%)				
	0 Day	15 Day	30 Day	45 Day	60 Day	0 Day	15 Day	30 Day	45 Day	60 Day	0 th Day	15 Day	30 Day	45 Day	60 Day
T₁	0.70	0.74 (0.04)	1.02 (0.28)	1.03 (0.01)	1.05 (0.02)	0.17	0.26 (0.09)	0.28 (0.02)	0.34 (0.06)	0.40 (0.06)	0.72	0.82 (0.10)	0.85 (0.03)	0.79 (0.06)	0.68 (0.11)
T₂	0.90	1.11 (0.21)	1.23 (0.12)	1.13 (0.10)	1.14 (0.01)	0.19	0.37 (0.18)	0.42 (0.05)	0.45 (0.03)	0.38 (0.07)	0.78	0.84 (0.06)	0.90 (0.06)	0.81 (0.09)	0.73 (0.08)
T₃	1.91	1.40 (0.51)	1.20 (0.20)	1.28 (0.08)	2.00 (0.72)	1.06	1.30 (0.24)	1.36 (0.06)	1.45 (0.09)	1.52 (0.07)	1.01	1.10 (0.09)	1.53 (0.43)	1.65 (0.12)	1.50 (0.15)
T₄	2.41	1.80 (0.61)	1.30 (0.50)	1.18 (0.12)	1.95 (0.77)	1.10	1.28 (0.18)	1.41 (0.13)	1.54 (0.13)	1.50 (0.04)	1.05	1.00 (0.05)	1.10 (0.10)	1.40 (0.30)	1.25 (0.15)
T₅	1.80	1.62 (0.18)	1.53 (0.09)	1.76 (0.23)	1.88 (0.12)	1.85	1.77 (0.08)	1.65 (0.12)	1.55 (0.10)	1.44 (0.11)	1.24	1.36 (0.12)	1.58 (0.22)	1.75 (0.17)	1.42 (0.33)
T₆	1.69	1.45 (0.24)	1.37 (0.08)	1.43 (0.06)	1.56 (0.13)	1.43	1.37 (0.06)	1.22 (0.15)	1.41 (0.19)	1.65 (0.24)	1.11	1.20 (0.09)	1.30 (0.10)	1.71 (0.41)	1.36 (0.35)
C.D.	0.107	0.072	0.046	0.084	0.090	0.080	0.064	0.042	0.090	0.069	0.069	0.058	0.046	0.103	0.064
SE(m)	0.034	0.023	0.015	0.027	0.029	0.026	0.020	0.014	0.029	0.022	0.022	0.019	0.015	0.033	0.021
C.V.	3.806	2.940	1.999	3.581	3.142	4.607	3.341	2.220	4.436	3.340	3.893	3.076	2.107	4.252	3.082

T₁-M₁-C₁- Composting without pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

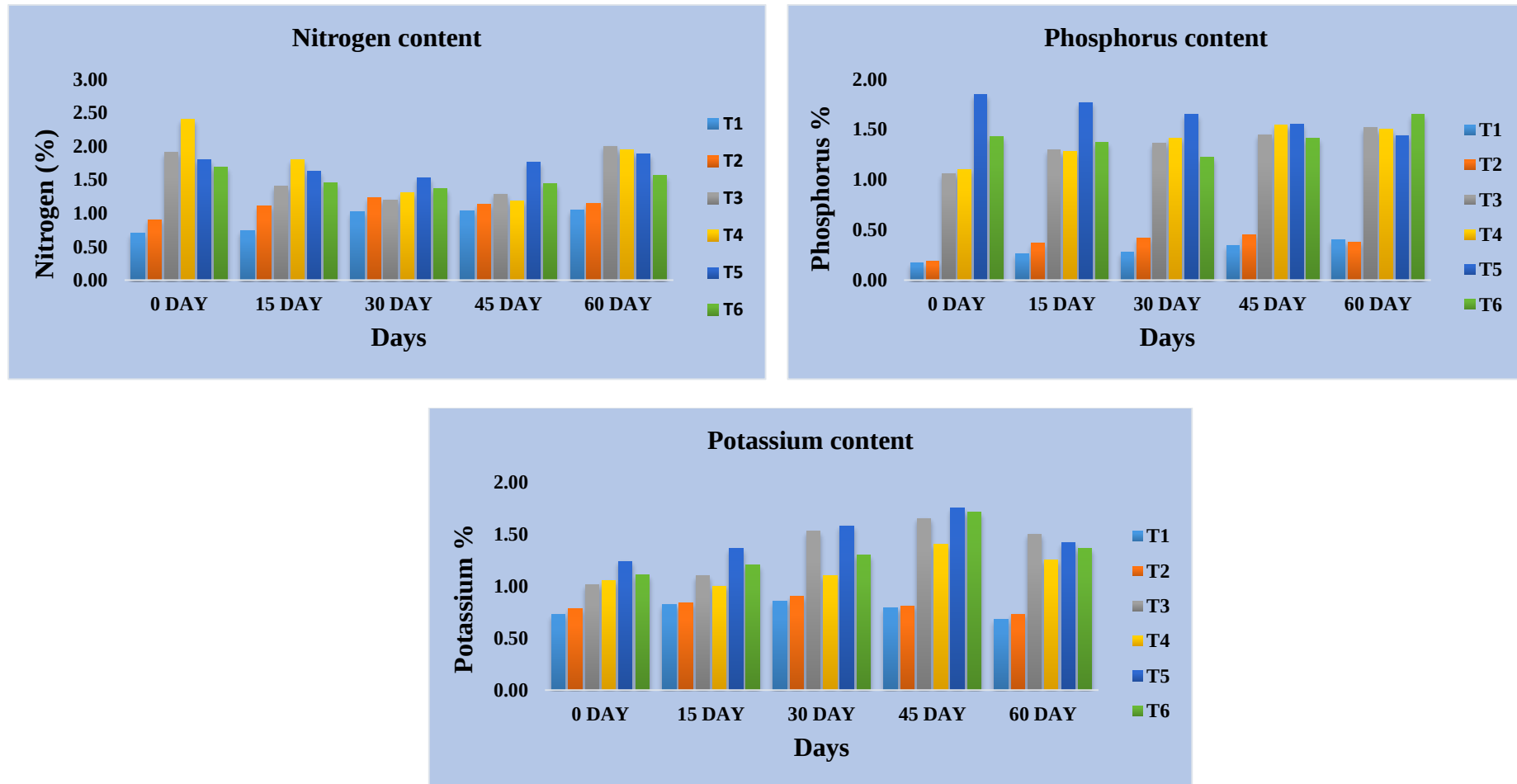
T₂-M₁-C₂- Composting with pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replication

Fig 4.9 Nitrogen, Phosphorus and Potassium content in different treatments during aerobic composting and anaerobic digestion of agricultural waste at different intervals.



compared to the observations of Patil *et al.* (2011) was studied and it obtained 0.22 % nitrogen, 0.2 % phosphorus and 1.27 % potassium.

The variation in the available potassium content between different treatments at different intervals of composting may be due to variation in chemical characteristics of substrates used and efficiency of organisms involved in degradation as similar kind of results were noticed in the study conducted by Chandrashekara *et al.* (2011) where, they have recorded more available potassium content in the compost prepared by using earth worms (0.61 per cent) compared to compost prepared by without inoculum (0.42 %) and also with microbial consortium (0.51 %).

4.2.3.4 Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

Organic carbon content of agricultural waste in the treatments was found on par with T₁ (Composting without pretreatment) (26.69 %), T₂ (Composting with pretreatment) (25.00 %) and T₃ (Vermicomposting without pretreatment) (22.00 %), T₄ (Vermicomposting with pretreatment) (23.50 %). Among all the treatments highest significant result was observed with the treatment T₆ (Biogas production with pretreatment) (i.e. 46.00 %) on 0th day followed by T₅ (Biogas production without pretreatment). Among different intervals organic carbon content was found to be increased up to 30th day except T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment). Gradual decreased in the organic carbon content was found from 0th day to 60th day in T₆ (Biogas production with pretreatment) (Table 4.13). Ideal organic carbon content in the compost and vermicompost was 38.00-40.00 % and 9.50-17.98 %. The variation in organic carbon content between different treatments at different intervals of composting, vermicomposting and biogas production may be due to variation in chemical characteristics of substrates used and efficiency of organisms involved in degradation

Organic carbon per cent in the T₆ (Biogas production with pretreatment) (41.9, 43.5 and 46.0 %) was more compared to the results reported by Yadav *et al.* (2013) who conducted experiments and showed results that indicate cow dung and biogas plant slurry can be used as a raw material in vermicomposting. The organic carbon content was 42.5 per cent and 40.0 per cent for biogas plant slurry.

BOD in the agricultural waste among all the treatments was found to be highest in the treatment T₆ (Biogas production with pretreatment) (106.80 mg l⁻¹) on 0th day.

Among treatments on par results were observed with the treatments of T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment) and on 60th day T₃ (Vermicomposting without pretreatment) and T₆ (Biogas production with pretreatment) were found to be on par. Among the intervals decrease in the BOD content was observed from 0th day to 60th day (Table 4.13). The ideal biological oxygen demand (100-110 mg l⁻¹) was important for the maintaining anaerobic conditions and production of methane under particular bioreactor by using agricultural waste as a substrate.

The similar kind of results were noticed in the study conducted by Sinha and Bharambe (2008) where, they have conducted an experiment on vermicompost by using earthworms and microorganisms and recorded biological oxygen demand as 63.5, 45.8 and 83.2 mg l⁻¹ in different treatments.

COD in the agricultural waste during aerobic composting and anaerobic digestion highest COD content was found on 15th day in T₂ (Composting with pretreatment) (199.85 g l⁻¹) followed by T₁ (Composting without pretreatment) (198.45 g l⁻¹). COD content was increased up to 15th day in all treatments except T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment). Among the treatments showing on par results with T₁ (Composting without pretreatment) to T₄ (Vermicomposting with pretreatment). Among different intervals drastic increase in the COD from 0th day to 15th day was recorded then gradual reduction in COD was observed from 30th day to 60th day (Table 4.13). The ideal chemical oxygen demand (145-150 g l⁻¹) was important for the maintaining anaerobic conditions and production of methane under particular bioreactor by using agricultural waste as a substrate.

The similar kind of results were noticed in the study conducted by Sinha and Bharambe (2008) where, they have conducted an experiment on vermicompost by using earthworms and microorganisms and recorded chemical oxygen demand as 112, 128, and 132 g l⁻¹ in different treatments.

Table 4.13. Contents of Organic carbon, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) in different treatments during aerobic composting and anaerobic digestion of agricultural waste at different intervals.

Agricultural waste	OC (%)					BOD (mg l ⁻¹)					COD (g l ⁻¹)				
	0 Day	15 Day	30 Day	45 Day	60 Day	0 Day	15 Day	30 Day	45 Day	60 Day	0 Day	15 Day	30 Day	45 Day	60 Day
T₁	26.69	30.00 (3.30)	36.50 (6.50)	30.10 (6.40)	26.50 (3.60)	95.90	83.20 (12.70)	63.50 (19.70)	60.48 (3.02)	45.80 (14.68)	101.30	198.45 (97.15)	195.20 (3.25)	193.45 (1.75)	190.82 (2.63)
T₂	25.00	26.40 (1.40)	28.10 (1.70)	36.50 (8.40)	31.32 (5.20)	97.20	86.50 (10.70)	64.20 (22.30)	66.40 (2.20)	60.54 (5.86)	99.20	199.85 (100.65)	180.45 (19.4)	186.75 (6.30)	180.59 (6.16)
T₃	22.00	28.50 (6.50)	32.40 (3.90)	26.53 (5.90)	24.60 (1.90)	99.10	94.20 (4.90)	91.50 (2.70)	90.50 (1.00)	89.40 (1.10)	98.79	168.25 (69.46)	165.42 (2.83)	160.75 (4.67)	158.47 (2.28)
T₄	23.50	26.20 (2.70)	29.40 (3.20)	28.16 (1.20)	22.52 (5.70)	98.20	89.40 (8.80)	85.10 (4.30)	80.70 (4.40)	79.80 (0.90)	97.23	160.78 (63.55)	158.89 (1.89)	155.23 (3.66)	150.47 (4.76)
T₅	35.20	32.30 (2.90)	30.60 (1.70)	33.46 (2.90)	30.65 (2.80)	101.20	90.67 (10.53)	51.82 (38.85)	80.16 (28.34)	49.41 (30.75)	110.30	107.50 (2.80)	62.33 (45.17)	94.70 (32.37)	59.46 (35.24)
T₆	46.00	43.50 (2.50)	41.90 (1.60)	35.10 (6.80)	31.26 (3.80)	106.80	95.02 (11.78)	81.21 (13.81)	84.75 (3.54)	90.65 (5.90)	109.60	103.60 (6.00)	90.30 (13.30)	95.30 (5.00)	84.66 (10.64)
C.D.	2.170	1.736	1.177	2.272	1.528	1.470	4.854	4.006	4.197	3.864	5.553	5.768	5.420	5.509	5.212
SE(m)	0.696	0.557	0.378	0.729	0.490	0.472	1.558	1.286	1.347	1.240	1.782	1.851	1.740	1.768	1.673
C.V.	4.057	3.099	1.974	3.991	3.054	0.821	3.004	3.056	3.024	3.101	3.005	2.050	2.120	2.074	2.109

T₁-M₁-C₁- Composting without pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

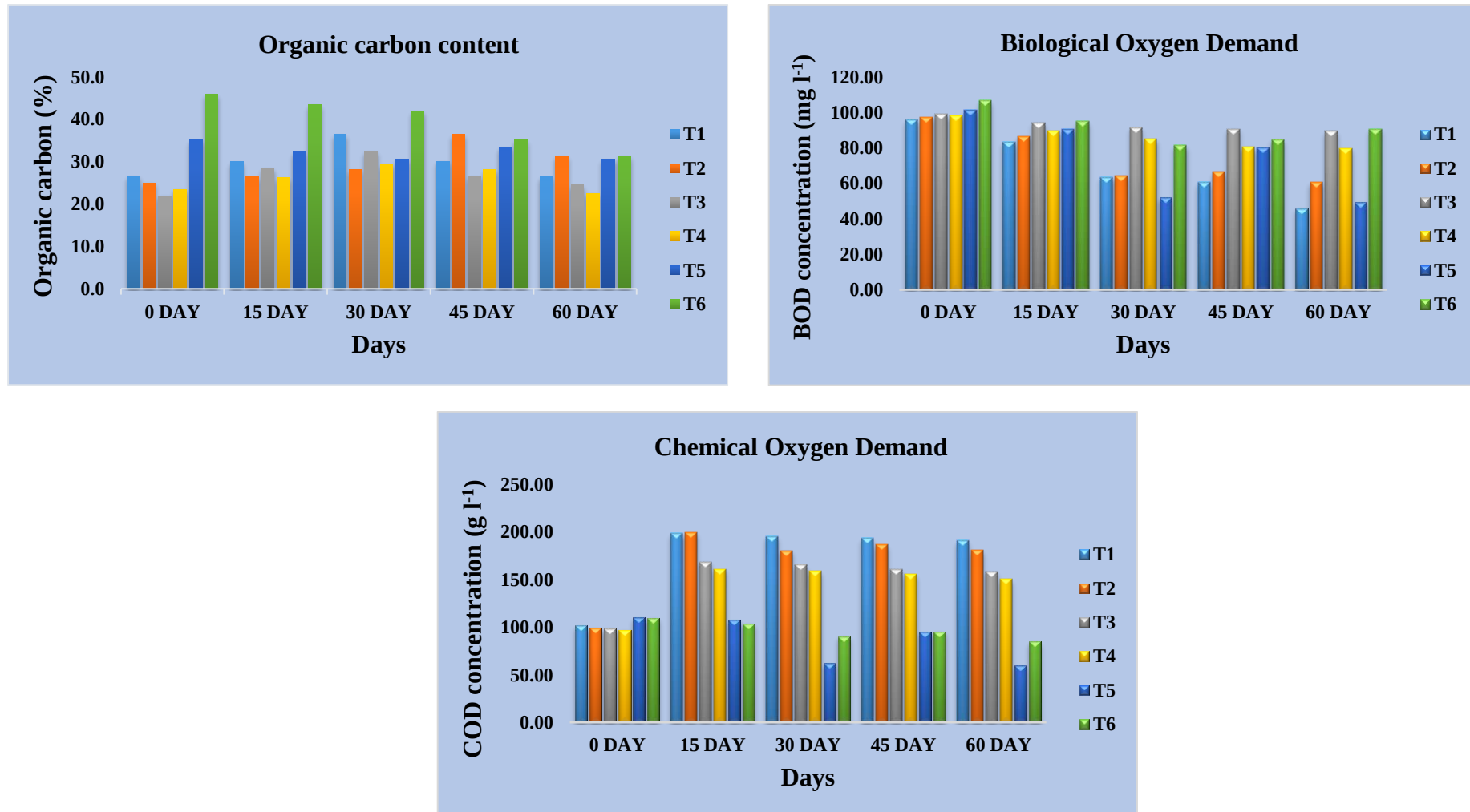
T₂-M₁-C₂- Composting with pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Fig 4.10 Contents of Organic carbon, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) in different treatments during aerobic composting and anaerobic digestion of agricultural waste at different intervals.



4.2.3.5 Cellulose content

At 0th day and 15th day the highest cellulose content was recorded in T₁ (Composting without pretreatment) (i.e. 25.06 %) followed by T₂ (Composting with pretreatment), T₃ (Composting without pretreatment) and lowest at T₆ (Biogas production with pretreatment). Among the treatments the results showed on par with T₁ (Composting without pretreatment), T₂ (Composting with pretreatment) and T₃ (Composting without pretreatment) followed by T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) 0th day followed by T₃ (Composting without pretreatment) and T₅ (Biogas production without pretreatment) on 60th day. In all the days of intervals T₆ (Biogas production with pretreatment) (12.85 %) treatment processes lowest cellulose content value (Table 4.14). Ideal cellulose content in compost was 36-39 % cellulolytic microorganisms can establish synergistic relationships with non-cellulolytic species in cellulosic wastes. The interactions between both populations lead to complete degradation of cellulose, releasing carbon dioxide and water under aerobic conditions, and carbon dioxide, methane and water under anaerobic conditions

The time of maximum cellulose degradation began when the composting material reached the thermophilic (53-63 °C) phase, and this increase in the rate of cellulose degradation coincided with the emergence of the actinomycetes as a major population. Cellulose per cent in the agricultural waste was similar (19.85 % in T₁ on 45th day), (12.85 % in T₆ on 45th day) and (23.02 % in T₁ on 0th day) to the results of Lin *et al.* (2014) who demonstrated that comparison of solid state anaerobic digestion and composting of yard trimmings with effluent from liquid anaerobic digestion. The cellulose per cent in the substrates were estimated as 19.1 % (in yard trimmings), 12.2 % (in leaves), and 23.8 % (in grass).

The variation in the percentage of cellulose present in the agricultural waste substrate between different treatments at different intervals may be due to variation in chemical characteristics of substrates used and organisms involved in degradation. Similar kind of result was noticed in the study by Chen *et al.* (2015) where, they have recorded cellulose content was 25.61 per cent.

Table 4.14. Cellulose content in different treatments in agricultural waste

Agricultural waste	0 Day (%)	15 Day (%)	30 Day (%)	45 Day (%)	60 Day (%)
T₁	23.02	25.06 (2.04)	20.05 (5.01)	19.85 (0.20)	16.20 (3.65)
T₂	22.98	23.86 (0.88)	21.45 (2.41)	20.05 (1.40)	15.98 (4.07)
T₃	22.05	20.40 (1.65)	17.40 (3.00)	16.89 (0.51)	17.26 (0.37)
T₄	21.10	20.25 (0.85)	22.65 (2.40)	18.52 (4.13)	18.02 (0.50)
T₅	16.00	16.89 (0.89)	17.38 (0.49)	15.58 (1.80)	16.39 (0.81)
T₆	15.85	13.54 (2.31)	14.75 (1.21)	13.05 (1.70)	12.85 (0.20)
C.D.	1.429	1.102	0.689	1.227	0.877
SE(m)	0.459	0.354	0.221	0.394	0.281
C.V.	3.936	3.064	2.021	3.934	3.024

AW- Agricultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

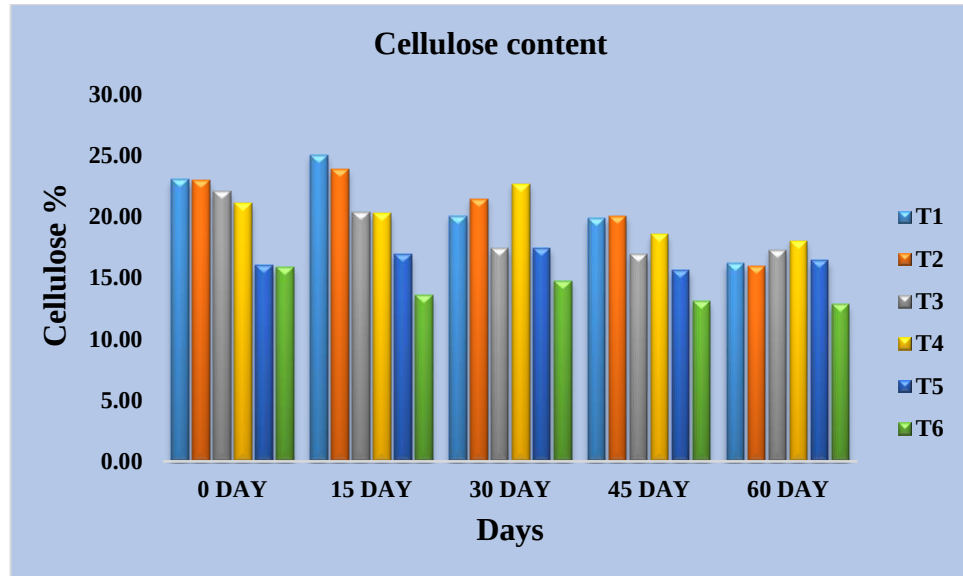
T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

*The table values with in the brackets indicate the difference between the values of adjacent days

Fig 4.11 Cellulose content in different treatments in agricultural waste



AW- Agricultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

4.2.3.6 Population of bacteria, fungi and actinomycetes

The population of bacteria, fungi and actinomycetes were fluctuated in all the treatments during aerobic composting process, however bacterial population in the substrate of agricultural waste among all treatments was found highest with the treatment T₁ (Composting without pretreatment) (8×10^6 CFU g⁻¹) followed by T₂ (Composting with pretreatment). Among different intervals gradual decrease in the population from 0th day to 60th day was observed in the treatment T₁ (Composting without pretreatment). In T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) treatments increase in the population from 0th day to 30th day was observed and later decreased gradually. Among different treatments fungal population was highest with the treatment T₆ (Biogas production with pretreatment) (4.5×10^4 CFU g⁻¹). Among different intervals the population decreased from 0th day to 60th day. Actinomycetes population was found to be highest in the treatment T₁ (Composting without pretreatment) (8×10^4 CFU g⁻¹) followed by T₂ (Composting with pretreatment) and lowest was observed in treatment T₅ (Biogas production without pretreatment). Among different intervals population was decreased from 0th day to 60th day in T₁ (Composting without pretreatment) and T₂ (Composting with pretreatment) followed by T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment) treatments. The population in the treatments of T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) was increased up to 45th day and slightly decreased on 60th day (Table 4.15). Ideal fungi and actinomycetes population in the process of vercomposting was 8×10^4 CFU g⁻¹ and 1×10^4 CFU g⁻¹ this will be mainly impact on the degradation of waste material and increase of nutrient status under suitable conditions of composting and vermicomposting.

Anaerobic digestion involved four different groups of microorganism namely hydrolytic bacteria, acidogens, acetogens, and methanogens with mutual dependence one to each other, particularly for energetic reason. The hydrolytic bacteria play a role in the first step by degrading complex organic matters into their monomers such as sugars, amino acids and fatty acids. The soluble monomers were then converted into short chain organic acid, acetic acid, alcohols, hydrogen and carbon dioxide by acidogen. The acidogenesis products were then subsequently converted into acetic acid by acetogens and finally converted into methane by methanogen. VFA has been suggested for a long time as one of the most important parameters in depicting the anaerobic digestion process.

Inhibition of the microbial activity at temperatures near 46 °C and pH below 6.0 is a common problem in the initial phase of agricultural waste composting. The variation in bacteria, fungal and actinomycetes population between different treatments at different intervals may be due to variation in chemical characteristics of substrates used and organisms involved in degradation. Similar kind of result was noticed in the study conducted by Chandrashekara *et al.* (2011) where, they have recorded a decrease in bacterial population at 12th to 35th days of composting in the compost prepared by different treatments. Similarly they have recorded more fungal population and less actinomycetes population in the compost prepared by using pretreated material than without pretreated material and earthworms at all stages of composting and vermicomposting.

The treatments contains a number of fungal species and greater population of other microbes, such as bacteria, protozoa, nematodes, actinomycetes which play an important role in organic matter decomposition by providing extra-cellular enzymes in the agricultural waste substrate (Suthar, 2010). This might be the reason for increased microbial population in the treatments of vermicompost with higher proportion of cow dung. This clearly indicates that the organic substrates used for composting and vermicomposting preparation could initiate the proliferation of the microorganisms and the earthworm species used for vermicomposting also acted as a medium for the rapid microbial colonization and thereby increasing the microbial activity.

Table 4.15. Population of bacteria, fungi and actinomycetes during aerobic composting and anaerobic digestion of agricultural waste

Agricultural waste	0 Day			15 Day			30 Day			45 Day			60 Day		
	B	F	A	B	F	A	B	F	A	B	F	A	B	F	A
T₁	8.00	2.70	8.00	7.60 (0.40)	2.60 (5.00)	7.50 (0.50)	7.20 (0.40)	2.50 (0.10)	7.00 (0.50)	7.00 (0.20)	2.40 (0.10)	6.80 (0.20)	6.40 (0.60)	2.10 (0.30)	6.10 (0.70)
T₂	6.00	3.90	6.50	6.50 (0.50)	3.80 (0.10)	6.40 (0.10)	6.20 (0.30)	3.50 (0.30)	6.30 (0.10)	6.00 (0.20)	3.10 (0.40)	6.00 (0.30)	5.80 (0.20)	3.00 (0.10)	5.80 (0.20)
T₃	4.00	4.10	5.00	4.00 (0.00)	4.20 (0.10)	5.20 (0.20)	4.30 (0.30)	4.00 (0.20)	5.40 (0.20)	4.20 (0.10)	3.80 (0.20)	5.00 (0.40)	3.90 (0.30)	3.20 (0.60)	4.80 (0.20)
T₄	4.10	3.80	5.90	4.20 (0.10)	3.70 (0.10)	6.00 (0.10)	4.00 (0.20)	3.60 (0.10)	5.80 (0.20)	3.80 (0.20)	3.50 (0.10)	5.40 (0.40)	3.50 (0.30)	3.00 (0.50)	5.10 (0.30)
T₅	4.00	3.90	3.60	4.20 (0.20)	3.80 (0.10)	3.80 (0.20)	4.50 (0.30)	3.70 (0.10)	4.00 (0.20)	4.00 (0.50)	3.20 (0.50)	4.10 (0.10)	3.60 (0.40)	3.10 (0.10)	4.00 (0.10)
T₆	3.80	4.50	3.90	3.90 (0.10)	4.40 (0.10)	4.00 (0.10)	4.00 (0.10)	4.30 (0.10)	4.20 (0.20)	3.60 (0.40)	4.00 (0.30)	4.40 (0.20)	3.50 (0.10)	3.80 (0.20)	4.20 (0.20)
C.D.	0.280	0.208	0.309	0.288	0.203	0.304	0.283	0.197	0.299	0.267	0.181	0.286	0.247	0.164	0.270
SE(m)	0.090	0.067	0.099	0.092	0.065	0.098	0.091	0.063	0.096	0.086	0.058	0.092	0.079	0.053	0.087
C.V.	3.123	3.024	3.132	3.157	3.011	3.081	3.124	3.045	3.052	3.112	3.013	3.011	3.091	3.010	2.997

AW- Agricultural waste (B: Bacteria (10^6 CFU g⁻¹); F: Fungi (10^4 CFU g⁻¹); A: Actinomycetes (10^4 CFU g⁻¹))

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

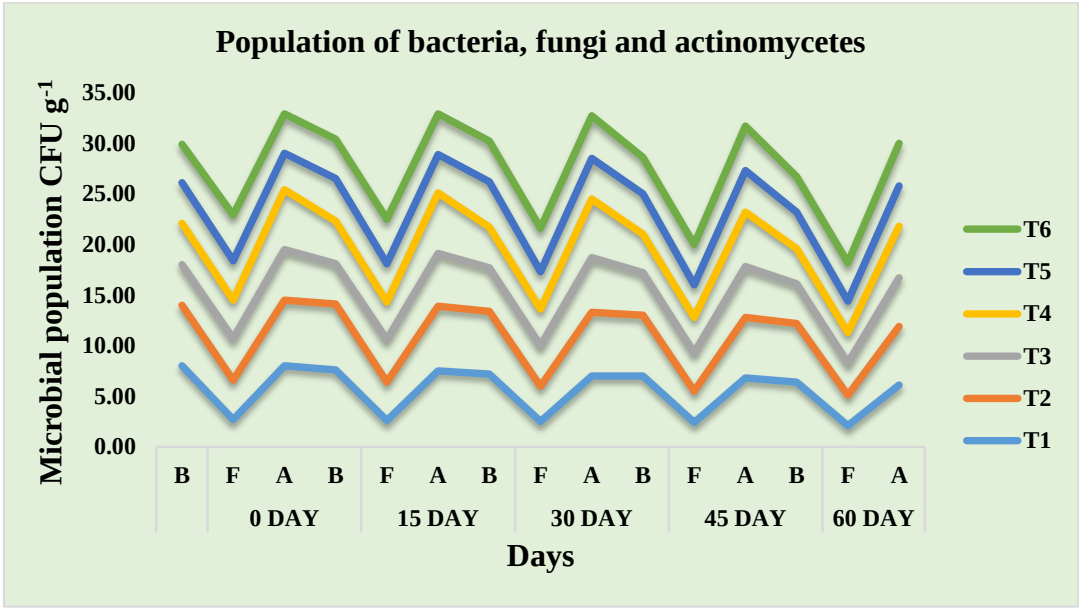
T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Fig 4.12 Population of bacteria, fungi and actinomycetes during degradation of agricultural waste



AW- Agricultural waste
T₁-M₁-C₁- Composting without pretreatment
T₂-M₁-C₂- Composting with pretreatment
T₃-M₂-C₁- Vermicomposting without pretreatment
T₄-M₂-C₂- Vermicomposting with pretreatment
T₅-M₃-C₁- Biogas production without pretreatment
T₆-M₃-C₂- Biogas production with pretreatment

4.3 ANALYSIS

4.3.1 Chemical Composition of Horticultural Waste

In horticultural waste TS % was found highest in T₆ (Biogas production with pretreatment) (13.25 %) which was on par with T₅ (Biogas production without pretreatment) and lowest TS % was observed in T₄ (Vermicomposting with pretreatment). TVS % was found highest in treatment T₆ (Biogas production with pretreatment) (86.35 %) which was on par with T₅ (Biogas production without pretreatment) followed by T₄ (Vermicomposting with pretreatment) which was on par with T₁ (Composting without pretreatment) and lowest was found in T₂ (Composting with pretreatment) (68.35 %). VFA was found highest in T₃ (Vermicomposting without pretreatment) (1.12 g l⁻¹) followed by T₁ (Composting without pretreatment) and lowest was found in T₄ (Vermicomposting with pretreatment). Alkaline pH content was found highest in T₄ (Vermicomposting with pretreatment) (8.34) which was on par with treatments T₁ (Composting without pretreatment), T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment) and least pH was found in T₅ (Biogas production without pretreatment). N content was observed highest in T₄ (Vermicomposting with pretreatment) (2.56 %) followed by T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) and least N content was recorded in the treatment T₁ (Composting without pretreatment). P content was found highest in T₅ (Biogas production without pretreatment) (1.60 %) followed by T₆ (Biogas production with pretreatment) and lowest was observed in T₁ (Composting without pretreatment). K content was found highest in the treatment T₆ (Biogas production with pretreatment) (1.13 %) which was on par with treatment T₅ (Biogas production without pretreatment). Organic carbon content was showed maximum in treatment T₆ (Biogas production with pretreatment) (40.90 %) followed by T₅ (Biogas production without pretreatment) and least organic carbon content was found in T₄ (Vermicomposting with pretreatment). BOD content was found highest in the treatment T₃ (Vermicomposting without pretreatment) (100.20 mg l⁻¹) which was on par with treatment T₁ (Composting without pretreatment), T₆ (Biogas production with pretreatment) and lowest was observed in T₅ (Biogas production without pretreatment). COD content was highest in T₄ (Vermicomposting with pretreatment) (110.60 g l⁻¹) which was on par with the treatment T₆ (Biogas production with pretreatment) and least COD content observed in T₂ (Composting with pretreatment) which was on par with T₅ (Biogas production without pretreatment). Cellulose percent was found highest in T₂ (Composting with pretreatment)

(25.38 %) which was on par with treatment T₄ (Vermicomposting with pretreatment) and least cellulose percent was observed in T₃ (Vermicomposting without pretreatment) (20.54 %) (Table 5.1).

In horticultural waste substrate treatments bacterial population significantly more in T₅ (Biogas production without pretreatment) (18×10^6 CFU g⁻¹) followed by T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment) and lowest bacterial count recorded in treatment T₁ (Composting without pretreatment). In the fungal population count highest in T₆ (Biogas production with pretreatment) (20×10^4 CFU g⁻¹) followed by T₅ (Biogas production without pretreatment), T₄ (Vermicomposting with pretreatment) and lowest fungal population observed in T₁ (Composting without pretreatment). Actinomycetes population count highest in T₁ (Composting without pretreatment) (10×10^4 CFU g⁻¹) followed by T₂ (Composting with pretreatment) and T₃ (Vermicomposting without pretreatment) and lowest actinomycetes population was observed in T₅ (Biogas production without pretreatment) (Table 5.2).

Chemical composition in the horticultural waste was similar to the results of Huang *et al.* (2016) who demonstrated that the optimal growth condition of earthworms and their vermicompost features during recycling of five different fresh fruit and vegetable wastes. The carbon and nitrogen % in the substrates were estimated as 31.8 % and 0.66 % in different fruit waste respectively.

Table 5.1 Chemical composition of different substrates at initial stage used in the treatments of Horticultural waste

Horticultural Waste	Total solids % (TS)	Total volatile solids % (TVS)	Volatile fatty acids g l⁻¹ (VFA)	pH	N %	P %	K %	Organic Carbon %	BOD mg l⁻¹	COD g l⁻¹	Cellulose %
T₁	11.36	79.85	0.84	8.25	0.75	0.10	0.60	29.20	98.00	99.30	23.01
T₂	10.59	68.35	0.76	8.15	0.85	0.15	0.65	26.80	92.00	90.00	25.38
T₃	12.35	72.45	1.12	8.28	1.00	1.16	1.00	25.30	100.20	98.50	20.54
T₄	10.56	80.12	0.48	8.34	2.56	1.10	0.99	19.80	95.40	110.60	24.03
T₅	13.07	85.20	0.60	6.19	1.78	1.60	1.10	38.50	90.50	92.30	23.28
T₆	13.25	86.35	0.68	6.29	1.46	1.46	1.13	40.90	98.40	109.60	23.68
C.D.	0.838	4.263	0.028	0.538	0.083	0.037	0.068	1.672	3.447	3.611	1.651
SE(m)	0.269	1.368	0.009	0.173	0.027	0.012	0.022	0.537	1.106	1.159	0.530
C.V.	3.923	3.010	2.117	3.940	3.286	2.242	4.153	3.091	2.001	2.007	3.934

HW- Horticultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Table 5.2 Population of bacteria, fungi and actinomycetes in horticultural waste

Horticultural waste			
RAW	Bacteria (10⁶ CFU g⁻¹)	Fungi (10⁴ CFU g⁻¹)	Actinomycetes (10⁴ CFU g⁻¹)
T₁	10.00	9.00	10.00
T₂	14.00	12.00	9.30
T₃	17.00	15.00	8.50
T₄	16.00	16.00	7.30
T₅	18.00	19.00	5.10
T₆	15.00	20.00	5.40
C.D	1.085	1.131	0.541
SE(m)	0.348	0.363	0.174
C.V	4.020	4.145	3.957

HW- Horticultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

4.3.2 Chemical Composition of Horticultural Waste Enriched With and Without Cellulose Degrading Bacteria

4.3.2.1 Contents of Total Solids (TS %), Total Volatile Solids (TVS %) and Volatile Fatty Acids (VFA)

The results revealed that the total solids during pretreatment of horticultural waste among the treatments of T₅ (Biogas production without pretreatment), T₆ (Biogas production with pretreatment) and T₂ (Composting with pretreatment), T₄ (Vermicomposting with pretreatment) was found on par except T₁ (Composting without pretreatment) and T₃ (Vermicomposting without pretreatment). Among all treatments the highest result was found on an average with the treatment T₆ (Biogas production with pretreatment) on 0th day and on par with the treatment T₅ (Biogas production without pretreatment) and lowest was observed with the treatment T₄ (Vermicomposting with pretreatment). Among different intervals gradual increase in the TS % was observed up to 14th day and gradually decreased was observed from 21st day to 28th day (Table 5.3). In biogas production from a slurry, the VS content of the material is an important as the TS content, since it represents the fraction of the solid material that may be transformed into biogas. Most manure and sludge from municipal wastes have a VS content of 70-90 % of the TS content. The Fixed Solids (FS, also termed the ash content) is comprised of inorganic material which dilute energy content and can impact the aerobic composting and anaerobic digestion.

TVS % among different treatments and intervals was found on par with the treatments T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment). Among all the treatments the highest TVS % was found with the treatment T₆ (Biogas production with pretreatment) which was found on par with the treatment T₅ (Biogas production without pretreatment) and found lowest with the treatment T₂ (Composting with pretreatment) on 0th day. Among different intervals TVS % was found increased up to 28th day in the treatments T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment), T₅ (Biogas production without pretreatment), T₁ (Composting without pretreatment) and T₄ (Vermicomposting with pretreatment) and T₆ (Biogas production with pretreatment) increase in the TVS % was observed up to 14th day and then decreased when it reached 28th day (Table 5.3). Although the VS content (i.e. 43-60.79 % for vermicompost and 86.3-91.6 % in biogas slurry) is an indicator of potential methane production, the specific methane yield on a VS basis is not a constant composition of the VS of the waste which includes both readily degradable organic

compounds including lipids, proteins, and carbohydrates, as well as more refractory organics which may include lignocellulose materials, complex lipopolysaccharides, structural proteins (keratin) and other refractory organics. This difference can be attributed to the high H : C ratio of lipids compared to carbohydrates. The complex nature of the composition of an organic waste means that the methane.

Among all the treatments VFA % was found highest in the treatment T₃ (Vermicomposting without pretreatment) (1.12 g l⁻¹) followed by T₁ (Composting without pretreatment) and T₂ (Composting with pretreatment) lowest was found in the treatment T₄ (Vermicomposting with pretreatment). Among different intervals on an average increase in VFA % was observed up to 7th day and later decreased to 28th day. On par results were observed with T₃ (Vermicomposting without pretreatment) (0.74 g l⁻¹) and T₆ (Biogas production with pretreatment) (0.75 g l⁻¹) on 28th day (Table 5.3). On cellulose-degrading bacteria, the appearance and increased amount of butyric acid and total VFA is due to increased cell wall hydrolysis i.e. better digestion of the substrate. Thus the early appearance of straight chain acids during digestion of vegetable market waste indicates its better digestion properties in comparison to horticultural waste.

The absolute values of TVS reductions were generally lower than those of the TS. This may be explained by the fact that volatile solids are converted to microbial biomass, Volatile Fatty Acids (VFA) and biogas. While microbial biomass contributes to the total solids content of the effluent. The diurnal cycling temperature also affected the TVS reductions, the higher the temperature the higher the TVS reduction. Unlike carbon and nitrogen, little or no significant losses in these inorganic compounds are generally expected to occur since they do not play a very significant role in the anaerobic digestion process, higher VFA concentrations were recorded at the lower temperature cycle.

Table 5.3. Total solids (%), Total Volatile Solids (%) and Volatile Fatty Acids in horticultural waste at different intervals.

Horticult ural waste	TS (%)					TVS (%)					VFA (g l⁻¹)				
	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day
T₁	11.36	15.62 (4.26)	31.50 (15.88)	29.45 (2.05)	16.59 (12.86)	79.85	81.25 (1.40)	85.37 (4.12)	83.60 (1.77)	80.45 (3.15)	0.84	0.85 (0.01)	0.62 (0.23)	1.65 (1.03)	0.93 (0.72)
T₂	10.59	12.56 (1.97)	15.42 (2.86)	18.50 (3.08)	17.48 (1.02)	68.35	70.41 (2.06)	74.23 (3.82)	78.15 (3.92)	81.62 (3.47)	0.76	0.78 (0.02)	0.75 (0.03)	0.83 (0.08)	0.81 (0.02)
T₃	12.35	13.52 (1.17)	15.74 (2.22)	15.02 (0.72)	16.03 (1.01)	72.45	74.26 (1.81)	76.48 (2.22)	79.26 (2.78)	82.43 (3.17)	1.12	0.43 (0.69)	1.41 (0.98)	1.40 (0.01)	0.74 (0.66)
T₄	10.56	12.03 (1.47)	13.87 (1.84)	14.52 (0.65)	15.89 (1.37)	80.12	82.56 (2.44)	83.26 (0.70)	80.17 (3.09)	79.89 (0.28)	0.48	0.52 (0.04)	0.47 (0.05)	0.73 (0.26)	0.85 (0.12)
T₅	13.07	14.63 (1.56)	16.85 (2.22)	15.25 (1.60)	14.17 (1.08)	85.20	85.89 (0.69)	86.02 (0.13)	86.78 (0.76)	87.58 (0.80)	0.60	0.45 (0.15)	0.53 (0.08)	0.72 (0.19)	0.79 (0.07)
T₆	13.25	15.85 (2.60)	16.23 (0.38)	14.21 (2.02)	13.49 (0.72)	86.35	87.56 (1.21)	88.59 (1.03)	85.37 (3.22)	83.43 (1.94)	0.68	0.73 (0.05)	0.75 (0.02)	0.65 (0.10)	0.75 (0.10)
C.D	0.838	0.740	1.020	1.000	1.105	4.263	4.360	4.460	4.425	4.457	0.028	0.035	0.042	0.055	0.032
SE(m)	0.269	0.238	0.327	0.321	0.355	1.368	1.400	1.432	1.420	1.431	0.009	0.011	0.014	0.018	0.010
C.V	3.923	2.932	3.104	3.117	3.933	3.010	3.018	3.012	2.992	3.001	2.117	3.124	3.106	3.093	2.210

T₁-M₁-C₁- Composting without pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

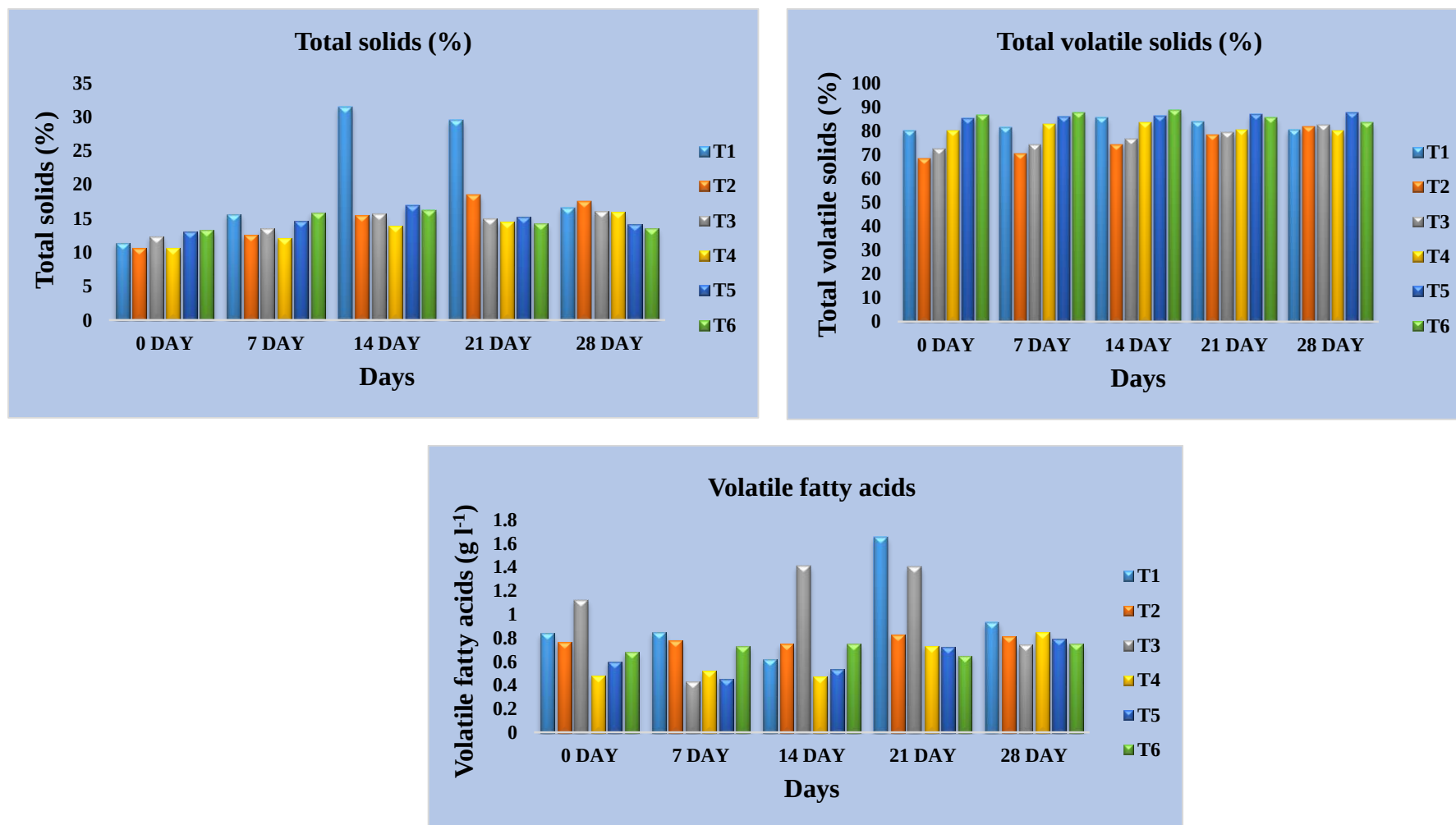
T₂-M₁-C₂- Composting with pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Fig 5.1 Contents of Total Solids (%), Total Volatile Solids (%) and Volatile Fatty Acids in horticultural waste with pretreatment at different intervals.



4.3.2.2 pH

The data showed that pH in the pretreated horticultural waste at initially (0th day) highest value recorded in T₄ (Vermicomposting with pretreatment) and lowest acidic pH value recorded at T₅ (Biogas production without pretreatment). At 7th day, 14th day, highest pH value recorded at T₄ (Vermicomposting with pretreatment) in all the intervals. In all the days of intervals increase in the pH was observed with the treatment T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment) from 0th day to 28th day. In the treatments T₁ (Composting without pretreatment), T₂ (Composting with pretreatment), T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) increase in the pH was observed from 0th day to 21st day (Table 5.4). The results showed that pH of the substrate has a significant effect on aerobic composting and anaerobic digestion, because it affects the activity of bacteria to destroy organic matter into biogas. A low pH in the composting and vermicomposting process inhibits the activity of microorganisms.

pH in the horticultural waste was higher (i.e. 8.15 on 0th day in T₂ to 8.50 on 28th day in T₄) when compared to the results of Irene *et al.* (2015) who studied that Opportunities and challenges of organic waste management from the agro industrial sector and recorded the pH of 6.07, 6.10 and 6.19 in different agro industrial waste at different intervals.

4.3.2.3 Contents of Nitrogen, Phosphorus and Potassium

Nitrogen content in different treatments measured in 5 times with one week interval. The results revealed that, initially in T₄ (Vermicomposting with pretreatment) (2.56 %) treatments N content is highest than other treatments. After 7 days interval N content was highest recorded in T₅ (Biogas production without pretreatment) treatments. The values of N content in 28th day was increased than 0th day. Lowest N content recorded T₁ (Composting without pretreatment) at 0th day. Increase in the N content was observed with all the treatments from 0th day to 21st day except T₅ (Biogas production without pretreatment). Phosphorus content recorded highest in T₅ (Biogas production without pretreatment) (i.e. 1.60 to 1.90 %) at all days of intervals 0, 7, 14, 21 and 28th followed by T₆ (Biogas production with pretreatment) in all intervals. Lowest values recorded in T₁ (Composting without pretreatment) on 0th day. Increase in the P content was observed with all the treatments from 0th day to 21st day except T₃ (Vermicomposting without pretreatment). Increase in the P content was continuous in case of T₅ (Biogas production

Table 5.4. pH in different treatments with pretreatment of horticultural waste

Horticultural waste	0 Day	7 Day	14 Day	21 Day	28 Day
T₁	8.25	8.29 (0.04)	8.38 (0.09)	8.48 (0.10)	8.45 (0.03)
T₂	8.15	8.18 (0.03)	8.25 (0.07)	8.30 (0.05)	8.25 (0.05)
T₃	8.28	8.32 (0.04)	8.35 (0.03)	8.37 (0.02)	8.39 (0.02)
T₄	8.34	8.37 (0.03)	8.40 (0.03)	8.45 (0.05)	8.50 (0.05)
T₅	6.19	6.26 (0.07)	6.30 (0.04)	6.34 (0.04)	6.12 (0.22)
T₆	6.29	6.33 (0.07)	6.38 (0.05)	6.43 (0.05)	6.33 (0.10)
C.D	0.538	0.417	0.417	0.417	0.544
SE (m)	0.173	0.134	0.134	0.134	0.174
C.V	3.940	3.039	3.019	2.998	3.936

HW- Horticultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

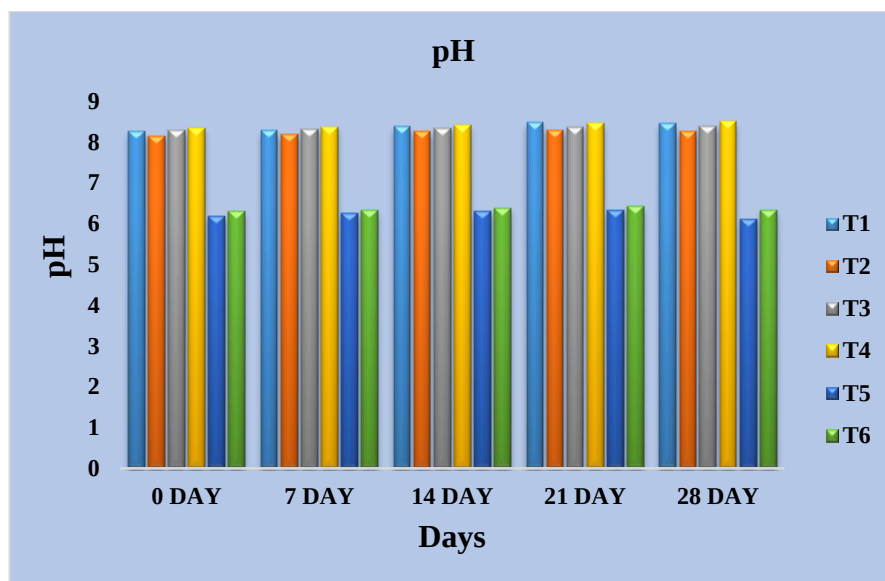
T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

*The table values with in the brackets indicate the difference between the values of adjacent days

Fig 5.2 pH in different treatments with pretreatment of horticultural waste



HW- Horticultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

without pretreatment) from 0th day to 28th day (i.e. 1.60-1.90 %). Potassium content recorded highest T₆ (Biogas production with pretreatment) (1.20 %) at 0th, 7th day, T₃ (Vermicomposting without pretreatment) at 14th day, T₅ (Biogas production without pretreatment) at 28th day and lowest K content was recorded in T₁ (Composting without pretreatment) treatment in all intervals of days. Increase in the K content was observed with all the treatments from 0th day to 21st day except T₃ (Vermicomposting without pretreatment). Continuous increase in K content was observed with T₅ (Biogas production without pretreatment) from 0th day to 28th day (Table 5.5). There was a significant variation in available nitrogen content in substrates between different treatments up to 21st day. This variation in available nitrogen content of substrates was noticed in all the stages of composting, vermicomposting and biogas production period. While nutrient content in a finished vermicompost certainly varies according to the initial feedstock, the nutrient content of the digested waste is often lower than that of the raw material. Some nitrogen loss is balanced by a reduction in volume, but the overall N content of a vermicomposted waste may be lower than the feedstock due to volatilization, leaching, or denitrification. Among all the treatments phosphorus content was a significantly variable and the amount of phosphorus content was increased in all substrates between different treatments up to 21st day. This variation in available phosphorus content of substrates was noticed in all the stages of composting, vermicomposting and biogas production period. The variation in the available potassium content between different treatments at different intervals of composting may be due to variation in chemical characteristics of substrates used and efficiency of organisms involved in aerobic composting and anaerobic digestion process. The most efficient composting occurs with an optimal C: N ratio of about 10:1 to 20:1. Rapid composting is favored by having a C/N ratio of 30 or less.

N, P and K % in the horticultural waste (1.62 % in T₆ (Biogas production with pretreatment), 0.17 % in T₁ (Composting without pretreatment) and T₂ (Composting with pretreatment) and 0.79 % in T₁ (Composting without pretreatment) and T₂ (Composting with pretreatment)) was similar to the results of Irene *et al.* (2015) who studied that Opportunities and challenges of organic waste management from the agroindustrial sector and recorded the N (1.61 %), P (0.13 %) and K (0.77 %) in different agroindustrial waste at different intervals.

Table 5.5. Nitrogen, Phosphorus and Potassium content in different treatments with pretreatment of horticultural waste at different intervals

Horticultural waste	N (%)					P (%)					K (%)				
	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day
T₁	0.75	1.20 (0.45)	1.35 (0.15)	1.68 (0.33)	0.80 (0.88)	0.10	0.17 (0.07)	0.24 (0.07)	0.26 (0.02)	0.12 (0.14)	0.60	0.72 (0.12)	0.79 (0.07)	0.81 (0.02)	0.68 (0.13)
T₂	0.85	0.90 (0.05)	1.13 (0.23)	1.15 (0.02)	0.90 (0.25)	0.15	0.17 (0.02)	0.34 (0.17)	0.37 (0.03)	0.20 (0.17)	0.65	0.74 (0.09)	0.79 (0.05)	0.82 (0.03)	0.70 (0.12)
T₃	1.00	1.11 (0.11)	1.23 (0.12)	1.24 (0.01)	1.10 (0.14)	1.16	1.20 (0.04)	1.25 (0.05)	1.22 (0.03)	1.19 (0.03)	1.00	1.16 (0.16)	1.20 (0.04)	1.14 (0.06)	1.10 (0.04)
T₄	2.56	1.16 (1.40)	1.24 (0.08)	1.25 (0.01)	2.80 (1.55)	1.10	1.15 (0.05)	1.18 (0.03)	1.20 (0.02)	1.12 (0.08)	0.99	1.02 (0.03)	1.06 (0.04)	1.09 (0.03)	1.05 (0.04)
T₅	1.78	1.79 (0.01)	1.83 (0.04)	1.78 (0.05)	1.82 (0.04)	1.60	1.62 (0.02)	1.69 (0.07)	1.79 (0.10)	1.90 (0.11)	1.10	1.14 (0.04)	1.15 (0.01)	1.17 (0.02)	1.20 (0.03)
T₆	1.46	1.56 (0.10)	1.62 (0.06)	1.65 (0.03)	1.59 (0.06)	1.46	1.52 (0.06)	1.58 (0.06)	1.60 (0.02)	1.52 (0.08)	1.13	1.16 (0.03)	1.18 (0.02)	1.20 (0.02)	1.17 (0.03)
C.D	0.083	0.071	0.076	0.079	0.086	0.037	0.064	0.067	0.067	0.042	0.068	0.049	0.056	0.056	0.072
SE (m)	0.027	0.023	0.024	0.025	0.027	0.012	0.021	0.022	0.022	0.014	0.022	0.016	0.018	0.018	0.023
C.V	3.286	3.060	3.016	3.015	3.171	2.242	3.687	3.575	3.486	2.326	4.153	2.735	3.024	2.994	4.094

T₁-M₁-C₁- Composting without pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

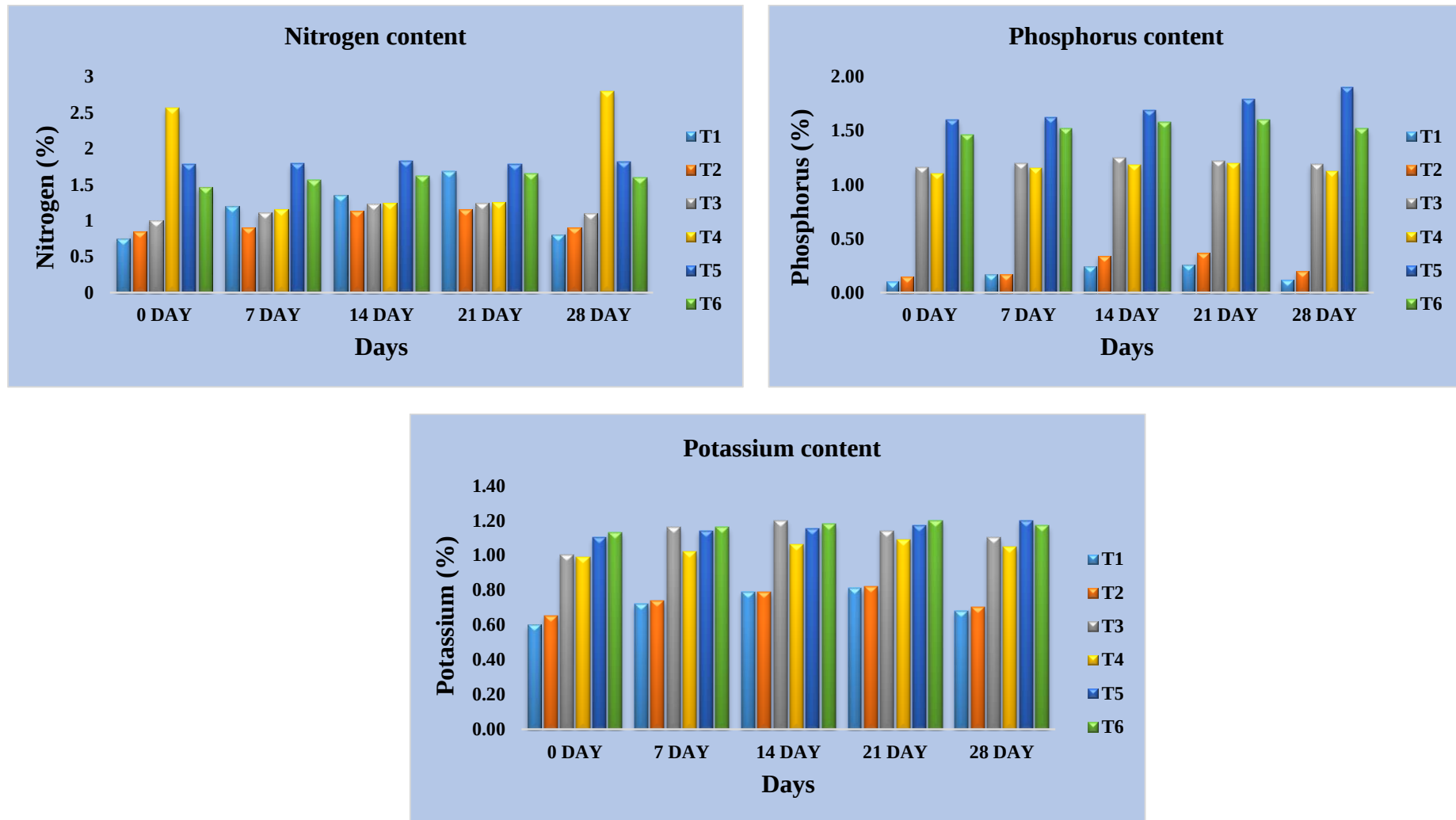
T₂-M₁-C₂- Composting with pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Fig 5.3 Nitrogen, Phosphorus and Potassium content in different treatments with pretreatment of horticultural waste at different intervals



4.3.2.4 Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

The results of organic carbon content in treatments during pretreatment of horticultural waste reveals that the highest organic carbon content observed in T₆ (Biogas production with pretreatment) (40.90 %) in all days of intervals. Lowest OC content was recorded in T₄ (Vermicomposting with pretreatment) treatments from 0 day to 28th day. Increase in the OC content was observed from 0th day to 21st day except in T₃ (Vermicomposting without pretreatment). OC content values of T₅ (Biogas production without pretreatment) treatment was on par with T₂ (Composting with pretreatment) and T₃ (Vermicomposting without pretreatment) on 0th day (Table 5.6). The relationships between the organic carbon and nitrogen degradation rates, as affected by substrate and microbial community C/N ratios and by the microbial conversion efficiency, the organic nitrogen mineralization rate constant is more complicated, since only a part of the degraded organic nitrogen was released as inorganic nitrogen in aerobic composting and anaerobic digestion.

The results of BOD indicated that the highest BOD values recorded in T₃ (Vermicomposting without pretreatment) (100.20 mg l⁻¹) treatment at 0th day. Lowest values recorded in T₅ (Biogas production without pretreatment) treatment at 0th day (90.50 mg l⁻¹). Increase in the BOD content was continuous from 0th day to 28th day in the treatments T₂ (Composting with pretreatment), T₄ (Vermicomposting with pretreatment), T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment). Decrease in the BOD content was observed with T₁ (Composting without pretreatment) and T₃ (Vermicomposting without pretreatment) from 0th day to 28th day. On par results was observed with the treatments T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment) on 28th day (Table 5.6). Dissolved oxygen depletion is most likely to become evident during the initial aerobic composting and anaerobic digestion, microbial population explosion in response to a large amount of organic material. If the microbial population deoxygenates the water, however, that lack of oxygen imposes a limit on population growth of microbial organisms.

The results of COD indicated that, the highest COD values recorded in T₄ (Vermicomposting with pretreatment) treatment on 0th day and T₆ (Biogas production with pretreatment) on 28th day. On par results were observed with the treatments T₄ (Vermicomposting with pretreatment) and T₆ (Biogas production with pretreatment).

Lowest value of COD recorded in T₂ (Composting with pretreatment) at 7th day, 21st day and T₄ (Vermicomposting with pretreatment) at 21st day and 28th days. Continuous increase in the COD value was observed with T₁ (Composting without pretreatment), T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment) and T₅ (Biogas production without pretreatment) (Table 5.6). The increasing COD indicated oxygen required to oxidized substance to carbon dioxide and water. As a result of this, COD values tend to be greater than BOD values. COD values can be vastly greater if large amounts of biologically resistant organic matter are present.

In the present study organic carbon % in the horticultural waste was less to the results of Irene *et al.* (2015) who studied that Opportunities and challenges of organic waste management from the agroindustrial sector and recorded the organic carbon (71.00, 80.80 and 8150 %) in different agroindustrial waste at different intervals.

BOD content in different treatments was similar to the results of Kavya *et al.* (2015) who studied that variations in BOD and COD at various stages of biogas production using different agricultural wastes and recorded the BOD (151.75 g l⁻¹, 80.14 g l⁻¹ and 76.26 g l⁻¹) in different treatments of agricultural waste digestion process.

In the present study COD content in the horticultural waste was higher when compared to the results of Jimenez *et al.* (2015) who studied that A new organic matter fractionation methodology for organic wastes: bio accessibility and complexity characterization for treatment optimization and recorded the COD (56.90, 79.50 and 98.20 g l⁻¹) in the digested sludge of agricultural waste in different treatments.

4.3.2.5 Cellulose content

The results of cellulose content in treatments of horticultural waste reveals that the T₂ (Composting with pretreatment) (i.e. 21.45 to 26.13 %) treatment samples shows significant maximum cellulose content in all days of intervals and lowest cellulose content was recorded in T₃ (Vermicomposting without pretreatment) at 0th day and 7th day, T₅ (Biogas production without pretreatment) at 14th day, T₆ (Biogas production with pretreatment) at 21st day. On par results were observed with T₂ (Composting with pretreatment) and T₄ (Vermicomposting with pretreatment) on 0th day and T₁ (Composting without pretreatment), T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment) on 28th day. Decrease in the cellulose content was continuous in case of T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) whereas

Table 5.6. Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments as pretreatment of horticultural waste at different intervals.

Horticultural waste	OC (%)					BOD (mg l ⁻¹)					COD (g l ⁻¹)				
	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day	0 Day	7 Day	14 Day	21 Day	28 Day
T₁	29.20	30.30 (1.10)	33.10 (2.80)	33.90 (0.80)	30.30 (3.60)	98.00	85.30 (12.70)	75.21 (10.09)	76.12 (0.91)	80.26 (4.14)	99.30	99.80 (0.50)	100.20 (0.40)	110.50 (1.01)	113.70 (3.20)
T₂	26.80	27.50 (0.70)	28.40 (0.90)	29.80 (1.40)	30.00 (0.20)	92.00	93.50 (1.50)	95.40 (1.90)	97.50 (2.10)	99.30 (1.80)	90.00	92.50 (2.50)	93.40 (0.90)	96.40 (3.00)	99.10 (2.70)
T₃	25.30	26.40 (1.10)	27.80 (1.40)	25.50 (2.30)	26.00 (0.50)	100.20	99.80 (0.40)	97.10 (2.70)	96.70 (0.40)	98.50 (1.80)	98.50	99.30 (0.808)	100.20 (0.90)	104.50 (4.30)	110.40 (5.90)
T₄	19.80	20.00 (0.20)	22.10 (2.10)	24.30 (2.20)	20.50 (3.80)	95.40	96.20 (0.80)	97.10 (0.90)	98.30 (1.20)	99.40 (1.10)	110.6 0	100.20 (10.40)	98.50 (1.70)	95.40 (3.10)	96.70 (1.30)
T₅	38.50	39.50 (1.00)	40.50 (1.00)	41.80 (1.30)	40.20 (1.60)	90.50	91.20 (0.70)	92.80 (1.60)	110.40 (17.6)	134.80 (2.24)	92.30	93.60 (1.30)	95.40 (1.80)	110.80 (15.4)	126.30 (15.5)
T₆	40.90	41.20 (0.30)	42.80 (1.60)	43.10 (0.30)	42.00 (1.10)	98.40	99.20 (0.80)	100.30 (1.10)	120.50 (20.20)	151.70 (3.20)	109.6 0	100.40 (9.20)	110.40 (0.10)	120.40 (10.06)	132.40 (12.00)
C.D	1.672	1.712	1.790	1.825	1.750	3.447	5.093	5.035	5.440	4.078	3.611	1.423	1.469	1.563	1.674
SE (m)	0.537	0.549	0.575	0.586	0.562	1.106	1.635	1.616	1.746	1.309	1.159	0.457	0.472	0.502	0.537
C.V	3.091	3.088	3.067	3.069	3.088	2.001	3.006	3.010	3.027	2.049	2.007	0.811	0.820	0.818	0.824

T₁-M₁-C₁- Composting without pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Fig 5.4 Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments as pretreatment of horticultural waste at different intervals.

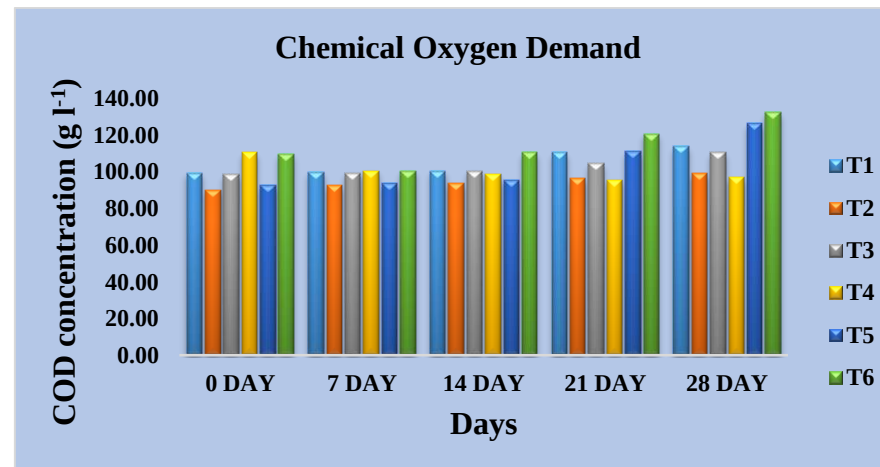
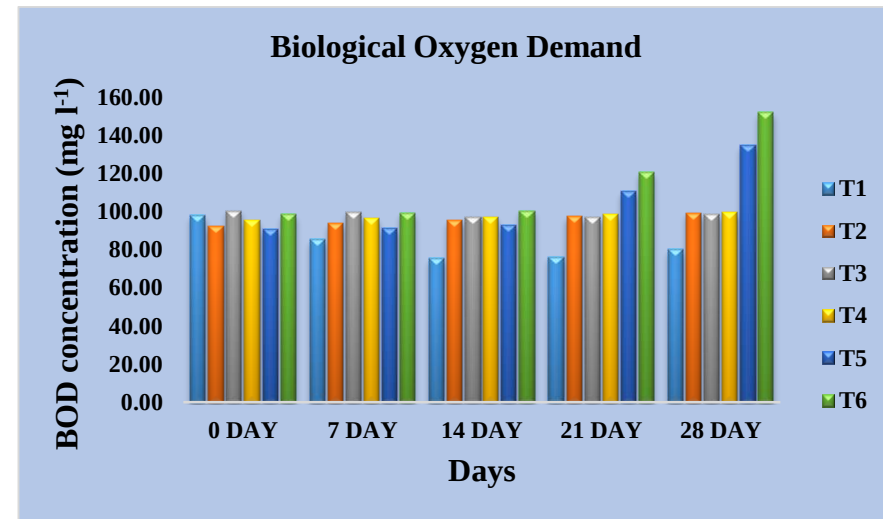
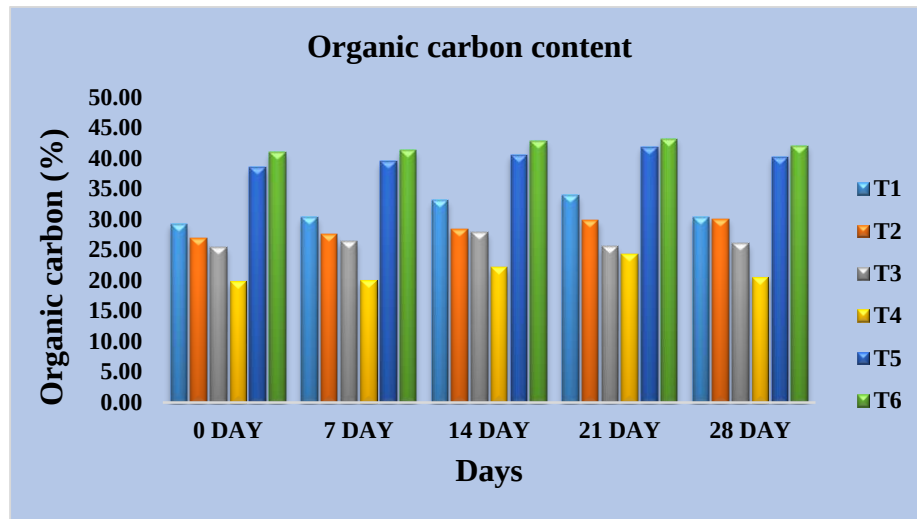


Table 5.7. Cellulose content in different treatments as pretreatment of horticultural waste

Horticultural waste	0 Day (%)	7 Day (%)	14 Day (%)	21 Day (%)	28 Day (%)
T₁	23.01	24.05 (1.04)	23.15 (0.90)	20.19 (2.96)	20.19 (0.00)
T₂	25.38	26.13 (0.75)	23.89 (2.24)	24.03 (0.14)	21.45 (2.58)
T₃	20.54	21.56 (1.02)	22.78 (1.22)	20.08 (2.70)	21.36 (1.28)
T₄	24.03	25.63 (1.60)	22.85 (4.07)	18.56 (4.29)	20.85 (2.29)
T₅	23.28	21.59 (1.69)	20.23 (1.36)	16.26 (3.97)	16.03 (0.23)
T₆	23.68	22.56 (1.12)	20.46 (2.10)	16.15 (4.31)	15.89 (0.26)
C.D	1.651	1.686	1.579	1.047	1.369
SE(m)	0.530	0.541	0.507	0.336	0.440
C.V	3.934	3.97	3.946	3.031	3.943

HW- Horticultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

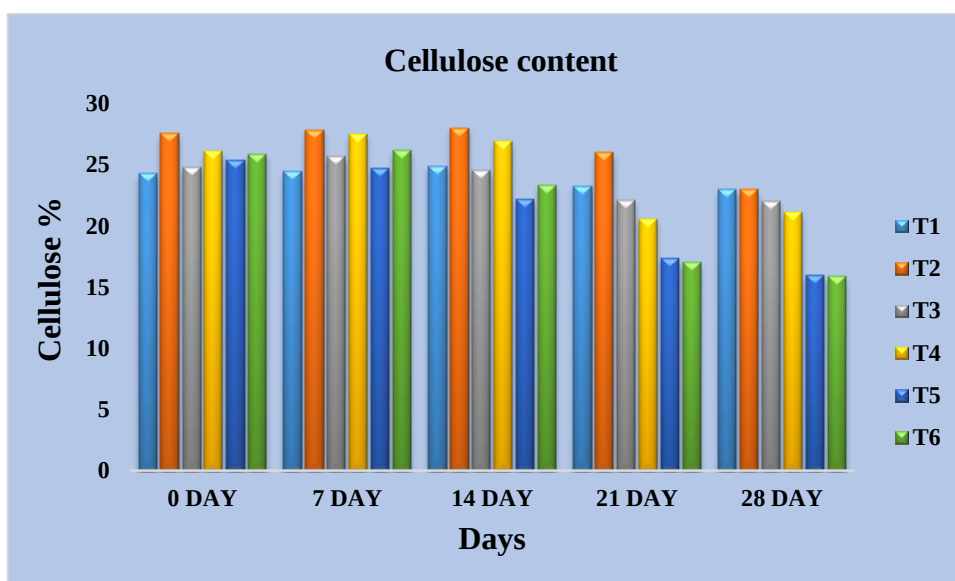
T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

*The table values with in the brackets indicate the difference between the values of adjacent days

Fig 5.5 Cellulose content in different treatments with pretreatment of agriculture waste



HW- Horticultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

it is discontinuous in case of T₁ (Composting without pretreatment), T₂ (Composting with pretreatment) and T₄ (Vermicomposting with pretreatment). Increase in the cellulose content was found in T₃ (Vermicomposting with pretreatment) from 0th day to 28th day (Table 5.7). Different habitats in which cellulose is widely available, by their differing characteristics (water availability, oxygen availability, redox potential, temperature variability, and nutrient status) have fostered the development of cellulose utilization strategies that differ in enzyme architecture and presentation, rate and extent of cellulolysis, ancillary hydrolytic activities, fate of hydrolytic products, and interactions among cellulolytic and non-cellulolytic microbes. Among the aerobic bacteria from compost, actinobacteria probably play a major role in the degradation of lignocelluloses. These bacteria can degrade cellulose and solubilize lignin. However, it seems that thermophilic and thermotolerant fungi, which are known to have cellulolytic and ligninolytic activity, are critical to the degradation of lignocellulosic materials in compost.

The overall percentage of cellulose was showing highest results in T₂ (Composting with pretreatment) (25.38, 26.13, 23.89, 24.03 and 21.45 %) among different intervals and also from different treatments. When compared with the results of Shankarappa *et al.* (2015) who studied the biological pretreatment of agroresidues with lignolytic fungi for delignification and recovery of cellulose and hemicellulose was showed cellulose content was 0.32 % in corn stover, 0.33 % in corn husk.

4.3.2.6 Population of bacteria, fungi and actinomycetes

The results of population of microorganisms revealing that at initial day 7th day, 14th day, 21th day and 28th day bacterial population was highest in T₅ (Biogas production without pretreatment) (i.e. 11 to 18 x 10⁶ CFU g⁻¹) on 0th day. Lowest bacterial population was recorded in T₁ (Composting without pretreatment) in all days of intervals. Bacterial population count was more at 0th day, compared to all the days of intervals. Continuous decrease in bacterial population was observed in all the treatments. Whereas fungal population was observed highest in T₆ (Biogas production with pretreatment) (16 to 20 x 10⁴ CFU g⁻¹) in all the days of intervals, lowest fungal population was recorded in T₁ (Composting without pretreatment) on 0th day. Fungal population was decreased from 0th day to 28th day in T₃ (Vermicomposting without pretreatment), T₄ (Vermicomposting with pretreatment), T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) whereas it is random in T₁ (Composting without pretreatment) and T₂ (Composting with pretreatment). Whereas actinomycetes population was observed highest in T₁ (Composting without pretreatment) (10 x 10⁴ CFU

g⁻¹) in all the days of intervals, lowest actinomycetes population was recorded in T₅ (Biogas production without pretreatment) Actinomycetes population was increased from 0th day to 28th day in T₁ (Composting without pretreatment) and T₂ (Composting with pretreatment) treatments whereas it is random in T₃ (Vermicomposting without pretreatment), T₄ (Vermicomposting with pretreatment) and T₆ (Biogas production with pretreatment). Increase in the actinomycetes population was observed in T₅ (Biogas production without pretreatment) from 0th day to 21st day (Table 5.8).

Fungi are less tolerant to high moisture conditions and are mainly decomposers of cellulose, chitin and lignin found at the later stages of composting. Therefore, the decreasing moisture contents and the presence of large cellulolytic materials in the systems might have made the number of fungi increase in the latter weeks of composting. The fall in the fungi numbers in composting systems might be due to faster and earlier decomposition of the cellulolytic materials. The inhibition of thermophiles at low pH is an important key to explain the often observed lag phase in the transition from mesophilic to thermophilic conditions in the initial phase of composting. The observed decrease in pH when the temperature was 46 °C and the initial pH was below 6.5 implies that in large-scale composting with limited cooling there is a risk that conditions with low degradation rates at low pH and high temperature are maintained for long periods of time. The microbial communities within an anaerobic digester treating animal manure are not fully characterized. However, due to the diversity of microorganisms in the system, a variety of methodological approaches are required for a detailed analysis of community structure in a bid to unravel the complex antagonistic and synergistic effects between microbial communities in order to eventually improve process stability and efficiency of aerobic composting and anaerobic digestion process. Microbial load in the substrates of treatments was similar to the results of Adegunloye *et al.* (2007) who demonstrated that microbial analysis of compost using cow dung as booster. The bacterial load in the substrates were estimated as 12 and 14 x 10⁶ CFU g⁻¹) from 5th week to 6th week intervals.

4.3.3 Aerobic Composting, Vermicomposting and Anaerobic Digestion of Horticultural Waste Enriched With and Without Cellulose Degrading Bacteria

4.3.3.1 Contents of Total Solids (TS %), Total Volatile Solids (TVS %) and Volatile Fatty Acids (VFA)

Among different treatments and intervals was found on par with the treatments T_1 (Composting without pretreatment), T_2 (Composting with pretreatment) on 0th day and T_2

Table 5.8. Population of bacteria, fungi and actinomycetes in different pretreated substrates of horticultural waste

Horticultural waste	0 DAY			7 DAY			14 DAY			21 DAY			28 DAY		
	B	F	A	B	F	A	B	F	A	B	F	A	B	F	A
T₁	10.00	9.00	10.00	9.50 (0.50)	9.50 (0.50)	9.60 (0.40)	9.20 (0.30)	9.80 (0.30)	9.40 (0.20)	8.70 (0.50)	8.50 (1.30)	9.00 (0.40)	8.20 (0.50)	8.00 (0.50)	8.50 (0.50)
T₂	14.00	12.00	9.30	10.40 (3.60)	11.90 (0.10)	9.20 (0.10)	9.50 (0.90)	11.20 (0.70)	8.60 (0.60)	8.70 (0.80)	10.45 (0.75)	8.40 (0.20)	6.30 (2.40)	10.02 (0.43)	8.00 (0.40)
T₃	17.00	15.00	8.50	15.00 (2.00)	8.90 (6.10)	7.30 (1.20)	12.32 (2.68)	13.24 (4.34)	8.45 (1.15)	11.20 (1.12)	12.04 (1.20)	8.12 (0.33)	10.12 (1.08)	11.41 (0.63)	7.50 (0.62)
T₄	16.00	16.00	7.30	14.05 (1.95)	15.78 (0.22)	7.50 (0.20)	12.05 (2.00)	14.02 (1.76)	7.80 (0.30)	10.48 (1.57)	13.63 (0.39)	7.00 (0.80)	9.65 (0.83)	12.76 (0.87)	6.50 (0.50)
T₅	18.00	19.00	5.10	15.48 (2.52)	18.96 (0.04)	5.40 (0.30)	14.85 (0.63)	17.52 (1.44)	5.80 (0.40)	12.56 (2.29)	16.53 (0.99)	6.20 (0.40)	11.52 (1.04)	15.02 (1.51)	6.00 (0.20)
T₆	15.00	20.00	5.40	13.56 (1.44)	19.75 (0.25)	5.60 (0.20)	12.45 (1.11)	18.65 (1.10)	5.00 (0.60)	12.03 (0.42)	17.52 (1.13)	4.80 (0.20)	11.46 (0.57)	16.03 (1.49)	4.50 (0.30)
C.D	1.085	1.131	0.541	0.932	1.060	0.541	0.819	1.024	0.523	0.734	0.961	0.505	0.674	0.903	0.487
SE(m)	0.348	0.363	0.174	0.299	0.340	0.174	0.263	0.329	0.168	0.236	0.309	0.162	0.216	0.290	0.156
C.V	4.020	4.145	3.957	3.981	4.168	4.045	3.878	4.043	3.866	3.845	4.076	3.871	3.926	4.113	3.963

HW- Horticultural waste (B: Bacteria (10^6 CFU g⁻¹); F: Fungi (10^4 CFU g⁻¹); A: Actinomycetes (10^4 CFU g⁻¹))

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

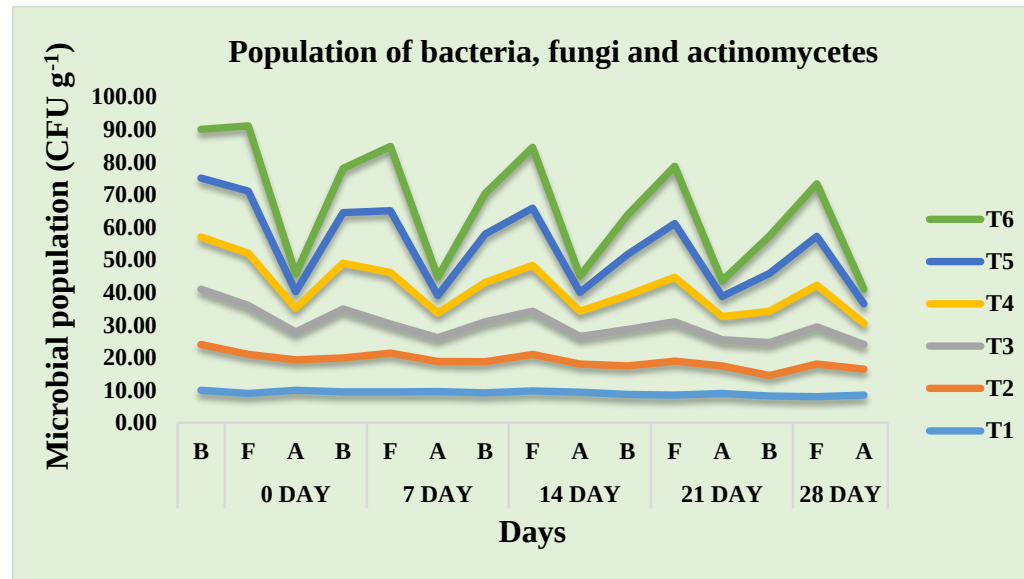
T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Fig 5.6 Population of bacteria, fungi and actinomycetes in different pretreated substrates of horticultural waste



HW- Horticultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

(Composting with pretreatment), T₅ (Biogas production without pretreatment) on 60th day. Among different treatments TS % was highest in T₂ (Composting with pretreatment) (17.42 %) followed by T₁ (Composting without pretreatment) and lowest was found with treatment T₆ (Biogas production with pretreatment). Among different intervals TS % gradually decreased from 0th day to 60th day. (Table 5.9). The total solids content of feedstocks affect the performances of aerobic composting and anaerobic digestion and the change of total solids content will lead to the change of microbial load in the treatments. As TS concentration increases, and slurries start acting as semi-solids, the method of expressing solid/liquid relationship of mixtures switches from solids content to moisture content. Most of the compost and vermicompost and biogas slurry contains TS % (16.9 %, 34.2 % and 10.5-12.5 %) by using agricultural waste as a substrate.

Total solids % in the horticultural waste (16.45 % in T₁ on 15th day) was similar to the results of Deressa *et al.* (2015) who demonstrated that the production of biogas from fruit and vegetable wastes mixed with cow manure in an anaerobic digester in a laboratory scale reactor at mesophilic conditions. The total solids per cent in the substrates were estimated as 16.40 % (in mango waste), 16.86 % (in tomato waste) and 19.00 % (in banana fruit waste).

Total volatile solids % in the horticultural waste among different treatments and intervals was found on par with the treatments T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) on 0th day. Whereas T₁ (Composting without pretreatment), T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment) on 60th day. Among different treatments T₅ (Biogas production without pretreatment) showed highest followed by T₆ (Biogas production with pretreatment) (83.43 %) and lowest was found with treatment T₄ (Vermicomposting with pretreatment). Among different intervals decreased in the TVS % was observed in all the treatments from 0th day to 60th day (Table 5.9).

Total volatile solids per cent in the horticultural waste was less (87.58 % in T₅ on 0th day and 86.43 % on 15th day) to the results of Deressa *et al.* (2015) who demonstrated that anaerobic digestion of fruit and vegetable waste in a laboratory scale reactor at mesophilic conditions (35 °C). The total volatile solids % in the substrates were estimated as 92.60 and 94.80 % in horticultural waste (banana and mango).

Total volatile solids per cent in the horticulture waste was similar (83.43 % in T₆ and 80.45 % in T₁ on 0th day) to the results of Velmurugan *et al.* (2011) demonstrated that

the total volatile solids per cent in the substrates, horticultural wastes were 83.10, 86.43 and 80.40 %. Total volatile solids (TVS) is the fraction of solid matter that can be oxidised and driven off as gas at 550 °C for 24 hours. This is an approximation, the organic fraction of the dry matter determined at 105 °C. The difference between the TS and TVS values is the inert (mineral) fraction, mainly due to inorganic matter. Most of the compost and vermicompost and biogas slurry contains TVS % (89.8, 43-60 and 86.3-91.6 %) by using agricultural waste as a substrate.

Volatile fatty acids concentration of horticultural waste among different treatments and intervals was found to be on par with the treatments T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) on 0th day, whereas T₁ (Composting without pretreatment) and T₂ (Composting with pretreatment) on 60th day. Among different treatments T₁ (Composting without pretreatment) (0.93 g l⁻¹) showed the highest VFA % followed by T₄ (Vermicomposting with pretreatment) and lowest was observed in T₃ (Vermicomposting without pretreatment). Among different intervals decrease in the VFA % from 0th day to 60th day except in T₆ (Biogas production with pretreatment). Final increase in the VFA content was observed in T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment). (Table 5.9). High Volatile Fatty Acid (VFA) concentrations in the aerobic composting and anaerobic digestion process will cause the inhibition of methanogenesis. Under conditions of overloading and in the presence of inhibitors, methanogenic activity cannot remove hydrogen and volatile organic acids as quickly as they are produced. The result is the accumulation of acids, the depletion of buffering capacity and the depression of pH to levels that also inhibit the hydrolysis/acidogenesis phase. It has also been shown that even when process pH is optimal, the accumulation of VFA may contribute to a reduced rate of hydrolysis of the solid organic substrate.

Volatile fatty acids per cent in the horticultural waste was similar to the results of Yangyang *et al.* (2016) who demonstrated that anaerobic digestion of corn stover in a laboratory scale reactor at mesophilic conditions (35 °C). The Volatile

Table 5.9. Total Solids (%), Total Volatile Solids and Volatile Fatty Acids content in aerobic composting and anaerobic digestion of horticultural waste at different intervals

Horticultural waste	TS (%)					TVS (%)					VFA (g l ⁻¹)				
	0 Day	15 Day	30 Day	45 Day	60 Day	0 Day	15 Day	30 Day	45 Day	60 Day	0 Day	15 Day	30 Day	45 Day	60 Day
T₁	16.59	16.45 (0.14)	16.02 (0.43)	15.78 (0.24)	15.18 (0.60)	80.45	79.88 (0.57)	78.45 (1.43)	77.14 (1.31)	76.02 (1.12)	0.93	0.90 (0.03)	0.88 (0.02)	0.85 (0.03)	0.70 (0.15)
T₂	17.48	17.42 (0.06)	17.06 (0.36)	16.89 (0.17)	16.15 (0.74)	81.62	80.98 (0.64)	79.85 (1.13)	78.15 (1.70)	77.93 (0.22)	0.81	0.79 (0.02)	0.75 (0.04)	0.70 (0.05)	0.68 (0.02)
T₃	16.03	15.98 (0.05)	15.45 (0.53)	15.14 (0.31)	14.99 (0.15)	82.43	81.30 (1.13)	80.12 (1.18)	79.84 (0.28)	78.72 (1.12)	0.74	0.73 (0.01)	0.68 (0.05)	0.65 (0.03)	0.60 (0.05)
T₄	15.89	15.75 (0.14)	15.62 (0.13)	15.03 (0.59)	15.07 (0.04)	79.89	76.56 (3.33)	75.42 (1.14)	74.12 (2.21)	73.21 (0.91)	0.85	0.83 (0.02)	0.78 (0.05)	0.72 (0.06)	0.62 (0.10)
T₅	14.17	12.79 (1.38)	8.48 (4.31)	10.46 (1.98)	6.99 (3.47)	87.58	86.43 (1.15)	56.34 (30.09)	80.40 (24.06)	53.41 (26.99)	0.79	0.52 (0.29)	1.02 (0.50)	0.71 (0.31)	1.19 (0.48)
T₆	13.49	12.76 (0.73)	10.98 (1.78)	9.49 (1.49)	8.58 (0.91)	83.43	85.42 (1.99)	82.13 (3.29)	80.46 (1.67)	74.48 (5.98)	0.75	0.48 (0.27)	0.69 (0.21)	0.78 (0.09)	1.25 (0.47)
C.D.	1.105	0.821	0.769	0.756	0.719	4.457	4.416	4.092	4.226	3.928	0.032	0.037	0.043	0.040	0.051
SE(m)	0.355	0.264	0.247	0.243	0.231	1.431	1.418	1.314	1.356	1.261	0.010	0.012	0.014	0.013	0.016
C.V.	3.933	3.005	3.068	3.047	3.116	3.001	3.003	3.018	2.999	3.021	2.210	2.939	2.976	2.991	3.367

T₁-M₁-C₁- Composting without pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

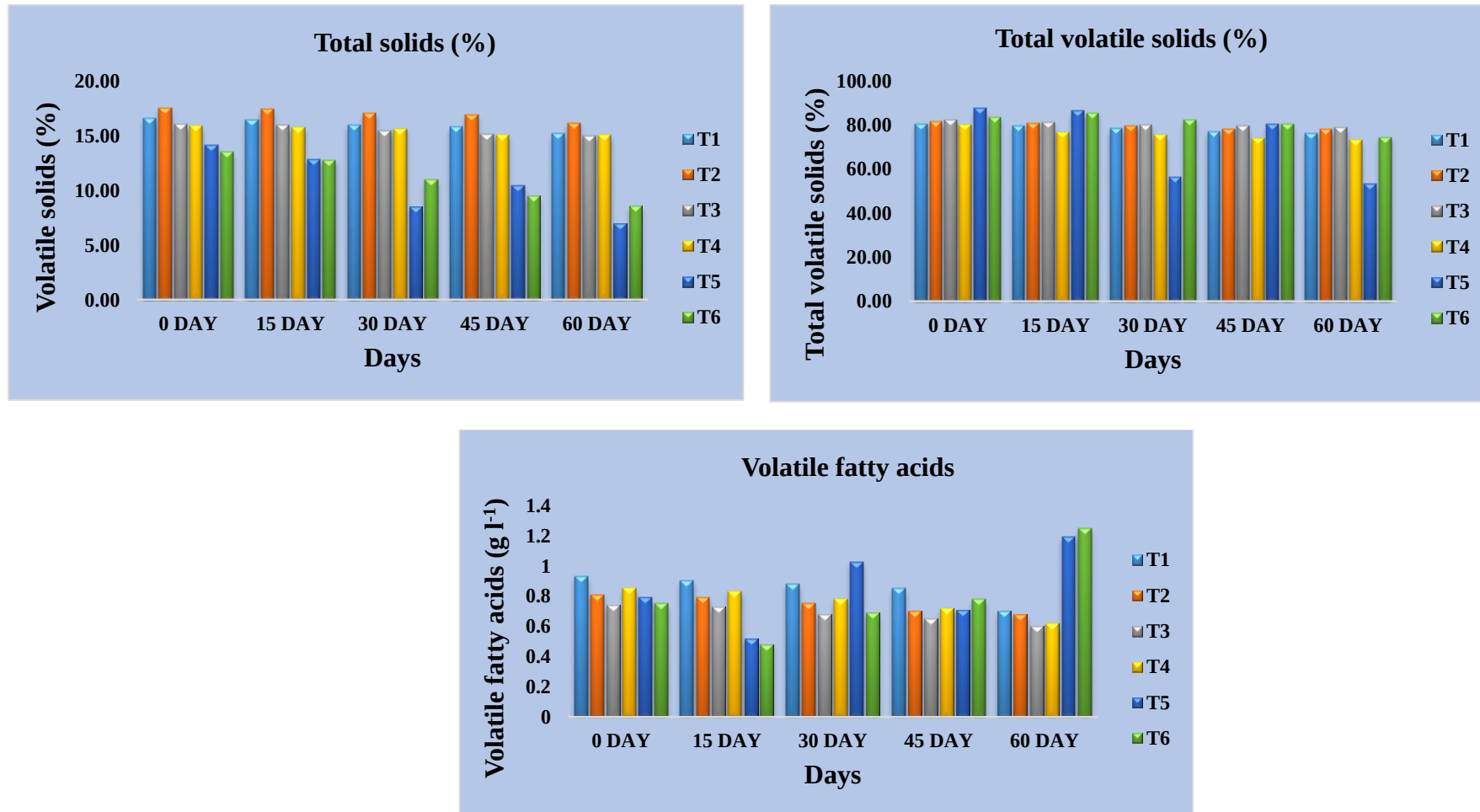
T₂-M₁-C₂- Composting with pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Fig 5.7 Total Solids (%), Total Volatile Solids and Volatile Fatty Acids in aerobic composting and anaerobic digestion of horticultural waste at different intervals



fatty acids per cent in the substrates were estimated as 0.64, 0.79 and 0.83 % in different treatments of agriculture waste (corn stover digestion).

4.3.3.2 pH

pH in samples at 0th day highest in T₄ (Vermicomposting with pretreatment) (8.50) and lowest in T₅ (Biogas production without pretreatment). At 15th day, 30th day highest pH in treatments T₁ (Composting without pretreatment) and T₅ (Biogas production without pretreatment). At 45th day highest pH value recorded in T₁ (Composting without pretreatment) and lowest in T₃ (Vermicomposting without pretreatment). At 60th day also highest pH value was recorded in T₁ (Composting without pretreatment) and lowest in T₄ (Vermicomposting with pretreatment). Initially alkaline pH was observed in the treatments T₁ (Composting without pretreatment), T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment). Whereas slightly acidic pH was observed in T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment). The final (60th day) pH in all the treatments was found to be near neutral (Table 5.10). Ideal pH for obtaining best compost was 7.1-8.3, and vermicompost is 7.2-7.9 this was varied from substrate to substrate and also different from the complete process of aerobic composting and anaerobic digestion process.

pH in the horticultural waste was similar to the results of Abubakar *et al.* (2012) who investigated the effectiveness of cow dung for biogas production and the pH of cow dung was in between 7.40 and 7.70 and during the anaerobic digestion there were variations between 6.12-7.70. The variation in pH between different treatments at different intervals of composting, vermicomposting and biogas production may be due to variation in chemical characteristics of substrates used and organisms involved in degradation as this was noticed in the study conducted by Chandrashekara *et al.* (2011) where, they have recorded more pH in the compost prepared by using earth worms (7.4) compared to compost prepared by without inoculums (6.5) and also with microbial consortium (6.8). pH recorded in the treatments of T₂ (Composting with pretreatment) and T₄ (Vermicomposting with pretreatment) was similar as 7.48 and 6.85.

Table 5.10. pH in the slurry samples

pH					
HW	0 Day	15 Day	30 Day	45 Day	60 Day
T₁	8.45	8.55 (0.10)	7.98 (0.57)	8.32 (0.34)	7.90 (0.42)
T₂	8.25	8.07 (0.18)	7.48 (0.59)	7.98 (0.50)	7.70 (0.28)
T₃	8.39	7.62 (0.77)	7.27 (0.35)	6.63 (0.87)	7.50 (0.87)
T₄	8.50	7.11 (1.39)	6.85 (0.26)	6.38 (0.47)	7.33 (0.95)
T₅	6.12	5.60 (0.52)	7.98 (2.38)	8.32 (0.34)	7.90 (0.42)
T₆	6.33	5.36 (0.97)	7.48 (2.12)	7.98 (0.50)	7.70 (0.28)
C.D.	0.162	0.393	0.149	0.315	0.301
SE(m)	0.052	0.126	0.048	0.101	0.097
C.V.	1.177	3.098	1.107	2.307	2.183

HW- Horticultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

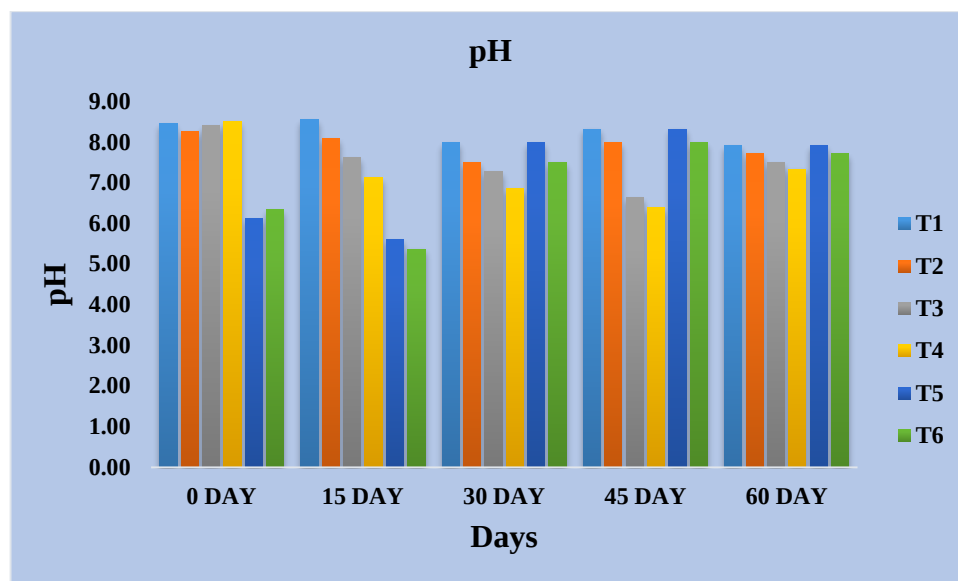
T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

*The table values with in the brackets indicate the difference between the values of adjacent days

Fig: 5.8 pH in the slurry samples



T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

4.3.3.3 Contents of Nitrogen, Phosphorus and Potassium

The available nitrogen in the horticultural waste among the treatments highest was found to be in T₄ (Vermicomposting with pretreatment) (i.e. 2.80 %) followed by T₅ (Biogas production without pretreatment) and lowest was found in treatment T₁ (Composting without pretreatment). Among different intervals increase in the N % was recorded in treatments T₁ (Composting without pretreatment) T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment) and T₅ (Biogas production without pretreatment) from 0th day to 60th day. In T₄ (Vermicomposting with pretreatment) decrease in the N % was observed up to 60th day. Increase in the N % was observed among all the treatments except T₄ (Vermicomposting with pretreatment). On par results was found among T₁ (Composting without pretreatment), T₂ (Composting with pretreatment), T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) on 0th and 60th day (Table 5.11).

The percentage of higher N per cent in T₅ (Biogas production without pretreatment) (1.82, 2.01 and 2.20 %) and T₆ (Biogas production with pretreatment) (2.07 and 2.30 %) from the present work as compared higher results compared to the work presented by Daicy *et al.* (2014) who studied the compost and vermicompost of horticultural waste as substrates for cutting rooting and growth of rosemary and estimated the composition of nutrients like N (0.14 and 0.15 % in compost) and (0.15 and 0.17 % in vermicompost).

The similar kind of results were noticed in the study conducted by Chandrashekara *et al.* (2011) where, they have recorded more phosphorus content in the compost prepared by using earth worms (1.90 %) compared to compost prepared by without inoculum (1.36 %) and also with microbial consortium (1.54 %).

Among all treatments highest significant P content was observed in T₅ (Biogas production without pretreatment) (i.e. 1.53 to 1.90 %) followed by T₆ (Biogas production with pretreatment) and lowest was recorded in T₁ (Composting without pretreatment) on 0th day. Among different intervals increase in P content up to 45th day observed in T₁ (Composting without pretreatment), T₂ (Composting with pretreatment), gradual increase in P content was observed up to 60th day in T₃ (Vermicomposting without pretreatment). Whereas gradual decrease in P content was observed in T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment). On par results were found with the treatments T₃ (Vermicomposting without pretreatment), T₄ (Vermicomposting

with pretreatment) on 0th day and T₅ (Biogas production without pretreatment), T₆ (Biogas production with pretreatment) on 60th day (Table 5.11).

The percentage of higher P per cent in T₅ (Biogas production without pretreatment) (1.90, 1.87 and 1.76 %) and T₆ (Biogas production with pretreatment) (1.52, 1.47 and 1.39 %) from the present work as compared with the results presented by Jeyabal *et al.* (2001) who studied the composition of nutrients like N, P and K in biogas digested slurry recorded as P (0.70, 0.76 and 1.20 %) in cow dung, biodigested slurry and sugarcane pressmud.

The nutrient states of higher P per cent in T₅ (Biogas production without pretreatment) (1.64 % in T₅ on 45th day) and T₆ (Biogas production with pretreatment) (1.39 % in T₆ on 30th day) from the present work as compared higher results compared to the work presented by Daicy *et al.* (2014) who studied the compost and vermicompost of horticultural waste as substrates for cutting rooting and growth of rosemary and estimated the composition of nutrients like P (0.03 and 0.04 % in compost) and (0.02, 0.03 and 0.04 % in vermicompost) in different treatments.

In the above result P in the treatments were similar to the observations of Ewemoje *et al.* (2016) who investigated the nutrients level of pit composting and vermicomposting and recorded the P in between 0.81 and 0.90 %.

Among all the treatments highest significant result was found with the treatment T₅ (Biogas production without pretreatment) followed by T₆ (Biogas production with pretreatment) and least was found with treatment T₁ (Composting without pretreatment). Among different intervals increase in the K content was observed up to 60th day in treatments T₄ (Vermicomposting with pretreatment) whereas T₃ (Vermicomposting without pretreatment), T₅ (Biogas production without pretreatment), T₆ (Biogas production with pretreatment) were increased upto 45th day. Increase in K content was found in T₁ (Composting without pretreatment), T₂ (Composting with pretreatment) up to 30th day. Final increase in the K content was observed among all the treatments except T₁ (Composting without pretreatment) and T₂ (Composting with pretreatment) (Table 5.11). The optimum N, P and K content in the compost (1.25, 0.25 and 2.00 %) and in vermicompost (2.10, 1.50 and 1.40 %) and in biogas slurry (3.4, 4.2 and 9.3 %).

In the results obtained as K % in the T₅ (Biogas production without pretreatment) was more (1.32, 1.45, 1.58 and 1.52 %) similar to the observations of Daicy *et al.* (2014) who studied the compost and vermicompost of horticultural waste as substrates for cutting

Table 5.11. Nitrogen, Phosphorus and Potassium content in different treatments during aerobic composting and anaerobic digestion of horticultural waste at different intervals.

Horticultural waste	N (%)					P (%)					K (%)				
	0 Day	15 Day	30 Day	45 Day	60 Day	0 Day	15 Day	30 Day	45 Day	60 Day	0 Day	15 Day	30 Day	45 Day	60 Day
T₁	0.80	0.92 (0.12)	1.20 (0.28)	1.25 (0.05)	1.36 (0.11)	0.12	0.32 (0.20)	0.40 (0.08)	0.42 (0.02)	0.38 (0.04)	0.68	0.72 (0.04)	0.85 (0.13)	0.78 (0.07)	0.62 (0.16)
T₂	0.90	1.09 (0.19)	1.20 (0.11)	1.23 (0.03)	1.30 (0.07)	0.20	0.37 (0.17)	0.46 (0.09)	0.41 (0.05)	0.31 (0.10)	0.70	0.75 (0.05)	0.80 (0.05)	0.72 (0.08)	0.60 (0.12)
T₃	1.10	1.22 (0.12)	1.29 (0.07)	1.45 (0.16)	1.90 (0.45)	1.19	1.25 (0.06)	1.30 (0.05)	1.47 (0.17)	1.50 (0.03)	1.10	1.24 (0.14)	1.36 (0.12)	1.58 (0.22)	1.42 (0.16)
T₄	2.80	2.00 (0.80)	1.80 (0.20)	1.90 (0.10)	1.98 (0.08)	1.12	1.33 (0.21)	1.49 (0.16)	1.40 (0.09)	1.58 (0.18)	1.05	1.16 (0.11)	1.20 (0.04)	1.40 (0.20)	1.56 (0.16)
T₅	1.82	1.60 (0.22)	1.49 (0.11)	2.01 (0.52)	2.20 (0.19)	1.90	1.87 (0.03)	1.76 (0.11)	1.64 (0.12)	1.53 (0.11)	1.20	1.32 (0.12)	1.45 (0.13)	1.58 (0.13)	1.52 (0.06)
T₆	1.59	1.42 (0.17)	1.32 (0.10)	2.07 (0.75)	2.30 (0.23)	1.52	1.47 (0.05)	1.39 (0.08)	1.43 (0.04)	1.51 (0.08)	1.17	1.26 (0.09)	1.39 (0.13)	1.45 (0.06)	1.36 (0.09)
C.D.	0.115	0.077	0.052	0.121	0.104	0.088	0.068	0.049	0.094	0.072	0.072	0.059	0.046	0.093	0.070
SE(m)	0.037	0.025	0.017	0.039	0.034	0.028	0.022	0.016	0.030	0.023	0.023	0.019	0.015	0.030	0.022
C.V.	4.248	3.128	2.107	4.085	3.154	4.825	3.437	2.416	4.622	3.547	4.094	3.062	2.170	4.115	3.282

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

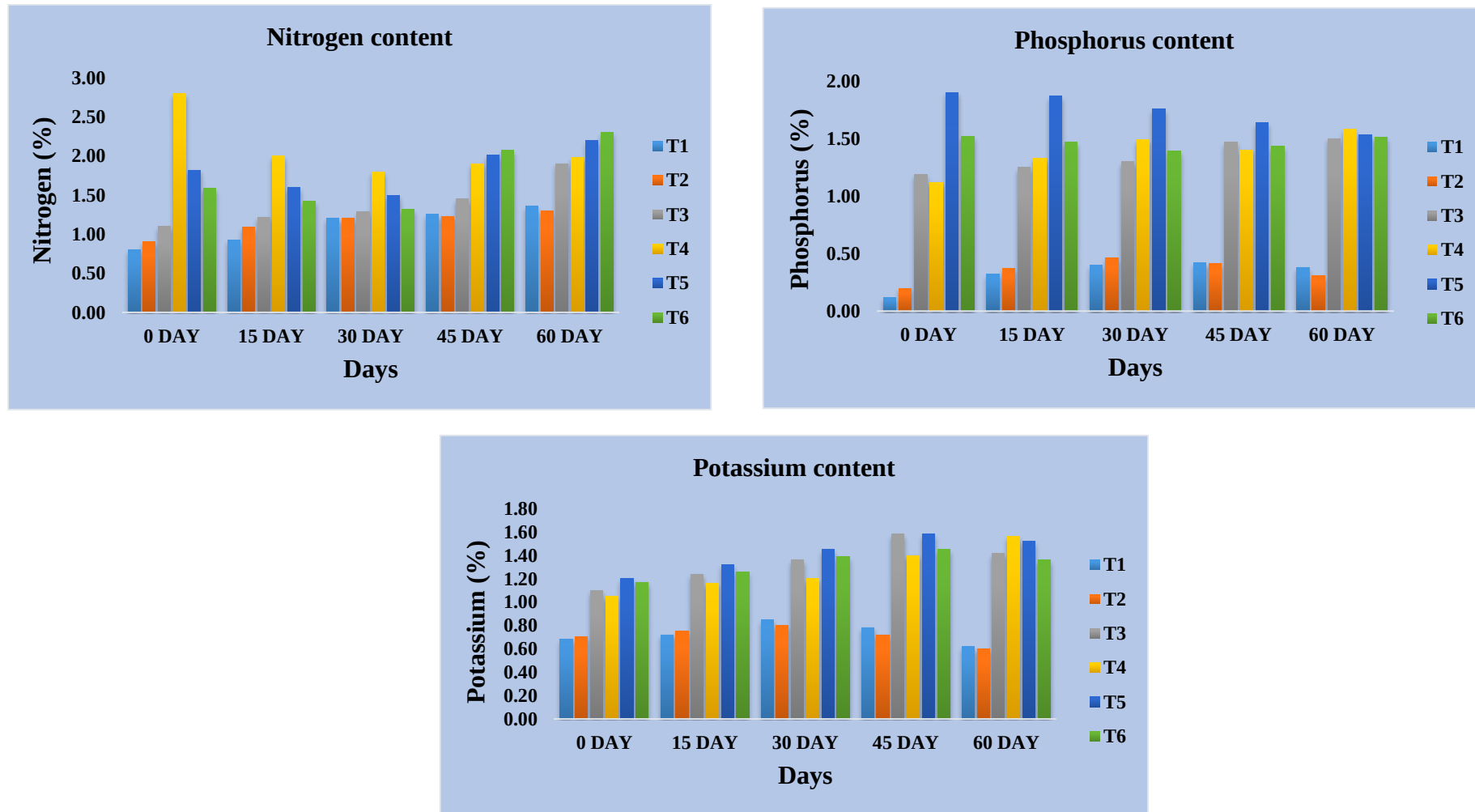
T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Fig 5.9 Nitrogen, Phosphorus and Potassium content in different treatments during aerobic composting and anaerobic digestion of horticultural waste at different intervals.



rooting and growth of rosemary and estimated the composition of nutrients during composting and vermicomposting and obtained 1.74 and 1.95 % of potassium in compost, 1.55, 1.57 and 1.62 % of potassium in different treatments of vermicompost.

The variation in the available potassium content between different treatments at different intervals of composting may be due to variation in chemical characteristics of substrates used and efficiency of organisms involved in degradation as similar kind of results were noticed in the study conducted by Chandrashekara *et al.* (2011) where, they have recorded more available potassium content in the compost prepared by using earth worms (0.61 %) compared to compost prepared by without inoculum (0.42 %) and also with microbial consortium (0.51 %).

In the above result K in the treatments were less to the observations of Ewemoje *et al.* (2016) who investigated the nutrients level of pit composting and vermicomposting and recorded the K in between 3.98 and 4.45 %.

4.3.3.4 Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

Organic carbon % in the horticultural waste among different treatments and intervals was found to be on par among the treatments T₅ (Biogas production without pretreatment), and T₆ (Biogas production with pretreatment). Among the treatments highest organic carbon content was observed in T₅ (Biogas production without pretreatment) (50.1 %) on 45th day which was on par with T₆ (Biogas production with pretreatment). Among the different intervals increase in organic carbon content up to 45th day was observed in treatments T₁ (Composting without pretreatment), T₄ (Vermicomposting with pretreatment), T₅ (Biogas production without pretreatment), and T₆ (Biogas production with pretreatment). Whereas increase in OC % from 0th day to 60th day was observed in T₃ (Vermicomposting without pretreatment), in case of T₂ (Composting with pretreatment) the increase in OC % was observed up to 30th day (Table 5.12). Ideal Organic carbon content in the compost (5-10 %) and in vermicompost (9-20 %) and in biogas slurry (45-54 %).

Organic carbon per cent in the T₅ (Biogas production without pretreatment) (46.2, 48.2, 50.1 and 45.7 %) and in T₆ (Biogas production with pretreatment) (44.5, 46.9 and 49.8 %) was more compared to the results reported by Ros *et al.* (2013) who conducted experiments anaerobic digestion (AD) from the waste products generated by the

processing of fruit and vegetables and showed results. The organic carbon content was 45.6 % in fruit and vegetable sludge.

Organic carbon per cent in the T₅ (Biogas production without pretreatment) (46.2, 48.2, 50.1 and 45.7 %) and in T₆ (Biogas production with pretreatment) (44.5, 46.9 and 49.8 %) was similar compared to the results reported by Choy *et al.* (2015) who conducted experiments on co-composting of horticultural waste with fruit peels, food waste, and soybean residues and showed results as 44.81 % (in horticultural waste) and 40.49 % (in fruit waste).

BOD in the horticultural waste among different treatments was found on par among the treatments T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment). Among the treatments highest significant result was found with the treatment T₆ (Biogas production with pretreatment) followed by T₅ (Biogas production without pretreatment) and lowest was found with the treatment T₁ (Composting without pretreatment) on 0th day. Whereas T₃ (Vermicomposting without pretreatment) was found to be highest and T₅ (Biogas production without pretreatment) was found to be lowest on 60th day. Among all the intervals gradual decrease in the BOD content was observed from 0th day to 60th day (Table 5.12). Biological oxygen demand is the amount of oxygen required for microbial metabolism of organic compounds in water. This demand occurs over some variable period of time depending on temperature, nutrient concentrations, and the enzymes available to indigenous microbial populations. The amount of oxygen required to completely oxidize the organic compounds to carbon dioxide and water through generations of microbial growth, death, decay, and cannibalism is total biological oxygen demand. The ideal biological oxygen demand (90-120 mg l⁻¹) was important for the maintaining anaerobic conditions and production of methane under specific bioreactor by using horticultural waste as a substrate.

The above results were similar to that of Liu *et al.* (2015) who studied on biodegradability of lignocellulosic residues and biogas generation from corn stover and corn bagasse obtained that the BOD content was 93.5 mg l⁻¹ and 99.4 mg l⁻¹.

COD in different treatments and intervals was found on par with the treatment T₁ (Composting without pretreatment) and T₂ (Vermicomposting with pretreatment) and T₄ (Vermicomposting with pretreatment) on 60th day. Among the treatments highest result on an average was found with treatment T₆ (Biogas production with pretreatment)

Table 5.12. Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments during aerobic composting and anaerobic digestion of horticultural waste at different intervals.

Horticultural waste	OC (%)					BOD (mg l ⁻¹)					COD (g l ⁻¹)				
	0 Day	15 Day	30 Day	45 Day	60 Day	0 Day	15 Day	30 Day	45 Day	60 Day	0 Day	15 Day	30 Day	45 Day	60 Day
T₁	30.3	33.9 (3.60)	39.5 (5.60)	42.3 (2.80)	31.2 (11.10)	80.26	79.45 (0.81)	78.12 (1.33)	77.32 (0.80)	75.02 (2.30)	113.70	105.42 (8.28)	103.88 (1.54)	97.82 (6.06)	95.45 (2.37)
T₂	30.0	39.4 (9.40)	40.1 (0.70)	27.3 (12.80)	23.2 (4.10)	99.30	83.65 (15.65)	82.25 (1.40)	81.75 (0.50)	80.26 (1.49)	99.10	110.78 (11.68)	102.85 (7.93)	98.75 (4.10)	96.25 (2.50)
T₃	26.0	30.5 (4.50)	32.6 (2.10)	36.5 (3.90)	40.0 (3.50)	98.50	90.78 (7.72)	89.62 (1.16)	87.78 (1.84)	85.15 (2.63)	110.40	99.75 (10.65)	98.25 (1.50)	94.26 (3.99)	93.02 (1.24)
T₄	20.5	27.9 (7.40)	30.4 (2.50)	32.2 (1.80)	23.3 (8.90)	99.40	87.23 (12.17)	85.26 (1.97)	84.17 (1.09)	83.46 (0.71)	96.70	100.12 (3.42)	98.65 (1.47)	97.28 (1.37)	95.35 (1.93)
T₅	40.2	46.2 (6.00)	48.2 (2.00)	50.1 (1.90)	45.7 (4.40)	134.80	107.60 (27.20)	63.94 (43.66)	83.80 (19.86)	49.45 (34.35)	126.30	118.60 (7.70)	61.25 (57.35)	78.60 (17.35)	59.39 (19.21)
T₆	42.0	44.5 (2.50)	46.9 (2.40)	49.8 (2.90)	38.5 (11.3)	151.70	103.60 (48.10)	81.88 (21.72)	81.30 (0.58)	74.60 (6.70)	132.40	128.60 (3.80)	95.40 (33.20)	85.40 (10.00)	80.25 (5015)
C.D.	2.280	2.016	1.458	2.869	1.868	4.078	4.998	4.350	4.464	4.078	1.674	3.994	3.396	3.307	3.201
SE(m)	0.732	0.647	0.468	0.921	0.600	1.309	1.604	1.396	1.433	1.309	0.537	1.282	1.090	1.062	1.027
C.V.	4.024	3.024	2.045	4.017	3.087	2.049	3.019	3.016	3.002	3.037	0.824	2.009	2.022	1.998	2.054

T₁-M₁-C₁- Composting without pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

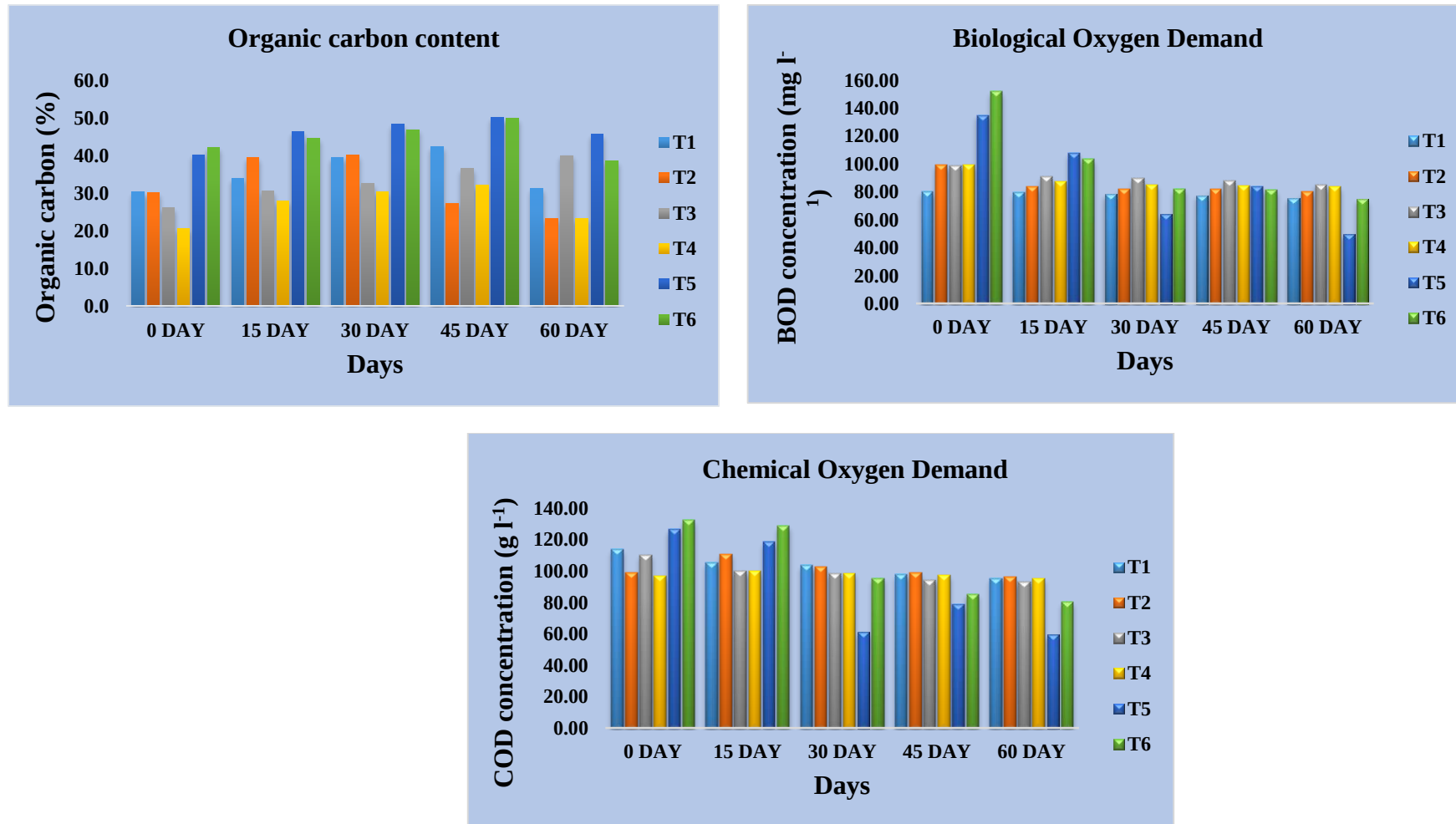
T₂-M₁-C₂- Composting with pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

Fig 5.10 Contents of Organic carbon, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in different treatments during aerobic composting and anaerobic digestion of horticultural waste at different intervals.



followed by T₅ (Biogas production without pretreatment) and lowest COD content was found with treatment T₄ (Vermicomposting with pretreatment). Among different intervals decrease in the COD content from 0th day to 60th day was observed (Table 5.12). Chemical Oxygen Demand (COD) is an important water quality parameter because, similar to BOD, it provides an index to assess the effect discharged wastewater will have on the receiving environment. Higher COD levels mean a greater amount of oxidizable organic material in the sample, which will reduce dissolved oxygen (DO) levels. A reduction in DO can lead to anaerobic conditions, which is deleterious to higher aquatic life forms. The COD test is often used as an alternate to BOD due to shorter length of testing time. The ideal chemical oxygen demand (97.8-156.3 g l⁻¹) was important for the maintaining anaerobic conditions and production of methane under specific bioreactor by using horticultural waste as a substrate.

The above results were similar to that of Liu *et al.* (2015) who studied on biodegradability of lignocellulosic residues and biogas generation from corn stover and corn bagasse obtained that the COD content was 115.00 g l⁻¹ and 118.00 g l⁻¹.

4.3.3.5 Cellulose content

Cellulose content in different treatments in horticultural waste revealed at different days of intervals. The results revealed that at 0th day, 30th day and 45th day highest cellulose content was recorded in T₂ (Composting with pretreatment) (22.08 %). At 15th day T₁ (Composting without pretreatment) treatment showed maximum (23.38 %) cellulose content, at 60th day T₃ (Vermicomposting without pretreatment) was recorded highest cellulose content (17.36 %) among the treatments. Cellulose content lowest recorded in T₆ (Biogas production with pretreatment) at all days of intervals. Gradual decrease in cellulose content was observed among the treatments T₅ (Biogas production without pretreatment) and T₆ (Biogas production with pretreatment) whereas random decrease was observed among the treatments of T₁ (Composting without pretreatment), T₂ (Composting with pretreatment), T₃ (Vermicomposting without pretreatment) and T₄ (Vermicomposting with pretreatment) (Table 5.13).

The above results were similar to that of Bouallagui *et al.* (2005) who studied on the potential of anaerobic digestion for material recovery and energy production from fruit and vegetable wastes and obtained that the cellulose % of fruit and vegetable waste substrates were 12.9 and 9.2 %.

Table 5.13. Cellulose content in different treatments in horticultural waste

Horticultural waste	0 Day (%)	15 Day (%)	30 Day (%)	45 Day (%)	60 Day (%)
T₁	20.19	23.38 (3.19)	19.89 (3.49)	18.98 (0.91)	16.48 (2.50)
T₂	21.45	22.08 (0.63)	20.79 (1.29)	19.56 (1.23)	15.06 (4.50)
T₃	21.36	22.36 (1.00)	17.01 (5.35)	16.52 (0.49)	17.36 (0.84)
T₄	20.85	21.48 (0.63)	18.56 (2.92)	17.01 (1.55)	16.56 (0.45)
T₅	16.03	15.88 (0.15)	17.05 (1.17)	15.38 (1.67)	15.32 (0.06)
T₆	15.89	15.09 (0.80)	14.74 (0.35)	14.58 (0.16)	14.03 (0.55)
C.D.	1.369	1.093	0.652	1.200	0.853
SE(m)	0.440	0.351	0.209	0.385	0.274
C.V.	3.943	3.033	2.013	3.922	3.003

HW- Horticultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

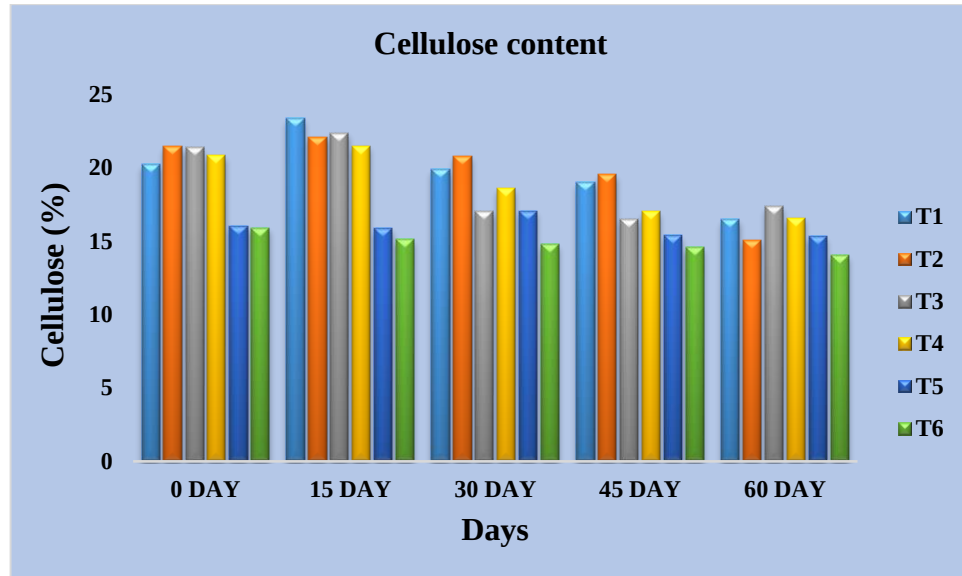
T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Values are mean of three replications

*The table values with in the brackets indicate the difference between the values of adjacent days

Fig 5.11 Cellulose content in different treatments in horticultural waste



HW- Horticultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

4.3.3.6 Population of bacteria, fungi and actinomycetes

Bacterial population in different substrates in horticultural waste among different treatments and intervals was found to be on par among all the treatments highest bacterial count was found highest in treatment T₅ (Biogas production without pretreatment) (11×10^6 CFU g⁻¹) followed by T₆ (Biogas production with pretreatment) and lowest was recorded in T₂ (Composting with pretreatment). Among different intervals gradual decrease in the population from 0th day to 60th day was observed in T₃ (Vermicomposting without pretreatment) to T₆ (Biogas production with pretreatment) (Table 5.14).

Fungal population count among different treatments and intervals was found to be highest in T₆ (Biogas production with pretreatment) followed by T₅ (Biogas production without pretreatment). Among different intervals gradual decrease in the population from 0th day to 60th day was observed in all the treatments. Among different treatments highest population was observed in T₆ (Biogas production with pretreatment) (16×10^4 CFU g⁻¹) followed by T₅ (Biogas production without pretreatment), and lowest was found with the treatment T₁ (Composting without pretreatment) (Table 5.14).

Actinomycetes population among different treatments highest significant result was observed in treatment T₁ (Composting without pretreatment) (8×10^4 CFU g⁻¹) followed by T₂ (Composting with pretreatment) and lowest was recorded in T₆ (Biogas production with pretreatment). Among different intervals gradually decrease in the population of actinomycetes was observed from 0th day to 60th day in all the treatments (Table 5.14). The variation in bacteria, fungal and actinomycetes population between different treatments at different intervals may be due to variation in chemical characteristics of substrates used and organisms involved in degradation. Similar kind of result was noticed in the study conducted by Chandrashekara *et al.* (2011) where, they have recorded a decrease in bacterial population at 12th to 35th days of composting in the compost prepared by different treatments. Similarly they have recorded more fungal population and less actinomycetes population in the compost prepared by using pretreated material than without pretreated material and earthworms at all stages of composting and vermicomposting. Due to the mesophilic transformation of compost, vermicompost contains greater microbial populations than thermophilic compost, leading to greater potential for odor reduction and nutrient mineralization

At the beginning of composting mesophilic bacteria predominate, but after the temperature increases to over 40 °C, thermophilic bacteria take over and thermophilic

fungi also appear in the compost. When the temperature exceeds 60 °C, microbial activity decreases dramatically, but after the compost has cooled mesophilic bacteria and actinomycetes again dominate. However, the accumulation of VFAs resulted in inhibition of the methane producing bacteria and hence reduction in biogas production. Organic wastes are first converted to long chain fatty acids in the acidogenic stage of anaerobic digestion processes. Volatile acids are further converted to acetic acid which together with hydrogen and formate serve as substrates for methane bacteria. Different species of anaerobic bacteria are involved in the conversion of specific long chain fatty acids to acetic acid. The increased proportion of propionic acid relative to acetic acid indicated that the different species of anaerobic bacteria did not respond in a similar manner to the effect of diurnally cyclic temperature. The fact that individual VFA are removed at different rates under adverse conditions such as a drop in temperature has been observed. A lower methane yield was recorded under the conditions in which the propionic acid concentration was high indicating that propionic acid was not available for conversion to methane. The treatments contains a number of fungal species and greater population of other microbes, such as bacteria, protozoa, nematodes, actinomycetes which play an important role in organic matter decomposition by providing extra-cellular enzymes in the agricultural waste substrate (Suthar, 2010). This might be the reason for increased microbial population in the treatments of vermicompost with higher proportion of cow dung. This clearly indicates that the organic substrates used for composting and vermicomposting preparation could initiate the proliferation of the microorganisms and the earthworm species used for vermicomposting also acted as a medium for the rapid microbial colonization and thereby increasing the microbial activity.

Table 5.14. Population of bacteria, fungi and actinomycetes in different substrates of horticultural waste

Horticultural waste	0 Day			15 Day			30 Day			45 Day			60 Day		
	B	F	A	B	F	A	B	F	A	B	F	A	B	F	A
T₁	8.20	8.00	8.50	8.30 (0.10)	7.80 (0.20)	8.40 (0.10)	7.90 (0.40)	7.50 (0.30)	8.00 (0.40)	7.50 (0.40)	7.20 (0.30)	7.50 (0.50)	7.00 (0.50)	7.00 (0.20)	7.20 (0.30)
T₂	6.30	10.02	8.00	6.50 (0.20)	9.80 (0.22)	7.50 (0.50)	6.30 (0.20)	9.50 (0.30)	7.00 (0.50)	6.00 (0.30)	9.00 (0.50)	6.50 (0.50)	5.70 (0.30)	8.50 (0.50)	6.10 (0.40)
T₃	10.12	11.41	7.50	9.80 (0.32)	10.03 (1.38)	7.20 (0.30)	8.70 (1.10)	9.80 (0.23)	6.80 (0.40)	7.50 (1.20)	9.50 (0.30)	5.80 (1.00)	7.20 (0.30)	9.02 (0.48)	5.50 (0.30)
T₄	9.65	12.76	6.50	9.12 (0.53)	11.00 (1.76)	6.10 (0.40)	8.50 (0.62)	9.40 (1.60)	5.90 (0.20)	8.00 (0.50)	9.10 (0.30)	5.50 (0.40)	7.40 (0.60)	8.70 (0.40)	5.00 (0.50)
T₅	11.52	15.02	6.00	10.86 (0.66)	14.20 (0.82)	5.50 (0.50)	10.25 (0.61)	13.85 (0.35)	5.12 (0.38)	10.00 (0.25)	12.02 (1.83)	4.80 (0.32)	9.46 (0.54)	11.00 (1.02)	4.20 (0.60)
T₆	11.46	16.03	4.50	10.59 (0.87)	15.20 (0.83)	4.10 (0.40)	10.13 (0.46)	14.62 (0.58)	4.00 (0.10)	10.03 (0.10)	10.53 (4.09)	3.80 (0.20)	9.80 (0.23)	9.53 (1.00)	3.70 (0.10)
C.D.	0.674	0.903	0.487	0.657	0.825	0.459	0.613	0.807	0.443	0.570	0.733	0.411	0.553	0.608	0.355
SE(m)	0.216	0.290	0.156	0.211	0.265	0.147	0.197	0.259	0.142	0.183	0.235	0.132	0.177	0.195	0.114
C.V.	3.926	4.113	3.963	3.967	4.047	3.943	3.947	4.159	4.009	3.880	4.263	4.041	3.957	3.775	3.729

HW- Horticultural waste (B: Bacteria (10^6 CFU g⁻¹); F: Fungi (10^4 CFU g⁻¹); A: Actinomycetes (10^4 CFU g⁻¹))

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

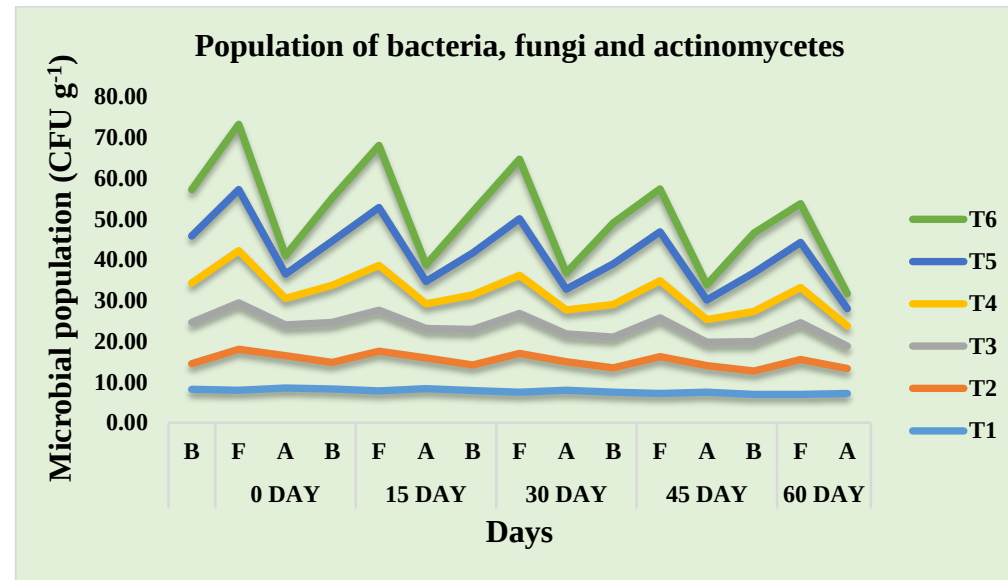
T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

Fig 5.12 Population of bacteria, fungi and actinomycetes in different substrates of horticultural waste



HW- Horticultural waste

T₁-M₁-C₁- Composting without pretreatment

T₂-M₁-C₂- Composting with pretreatment

T₃-M₂-C₁- Vermicomposting without pretreatment

T₄-M₂-C₂- Vermicomposting with pretreatment

T₅-M₃-C₁- Biogas production without pretreatment

T₆-M₃-C₂- Biogas production with pretreatment

4.4 PRODUCTION OF BIOGAS AND BIOETHANOL BY ANAEROBIC DIGESTION

Anaerobic digestion of agricultural and horticultural wastes were used for the production of biogas and bioethanol with and without pretreatment of substrates with efficient cellulose degrading cultures. All parameters such as TS %, TVS %, VFA, pH, N, P, K, Organic carbon, BOD, COD, cellulose and microbial population count were discussed under sections of 4.2 and 4.3, data on the production of biogas and bioethanol presented and discussed under this section.

4.4.1 Biogas Production from Agricultural and Horticultural Waste Enriched With and Without Cellulose Degrading Bacteria

All the biogas production units with four treatments and three replications were set on the same day with 250 grams cow dung, 500 grams substrate and 1000 ml water (1:2:5 ratio).

The results of biogas production revealed that, the end of the 7th day in AW-T₂ (Biogas production with pretreatment) 680.30 ml of biogas was released followed by (550.00 ml) in AW-T₁ (Biogas production without pretreatment). At the end of 15th day 720.30 ml of biogas was released in AW-T₁ (Biogas production without pretreatment) and lower amount was observed in HW-T₁ (Biogas production without pretreatment) (580.30 ml). At the end of 30th day in AW-T₁ (Biogas production without pretreatment) more amount of biogas was evolved (620.30 ml). At the end of 60th day highest gas production was observed in AW-T₂ (Biogas production with pretreatment) (580.00 ml). Based on the water displacement readings more biogas evolved in AW-T₁ (Biogas production without pretreatment) at 15th day (550.00 ml), 30th day (980.20 ml), AW-T₂ (Biogas production with pretreatment) having more biogas evolution at 7th day (680.30 ml), 45th day (620.30 ml) and 60th day (580.00 ml) (Table 5.15). Biogas production resumption time was longer with longer low temperature duration; and increased rapidly, then decreased slightly when temperature was restored in the low temperature duration of 12 h and 24 h. The delay in recovery was presumably due to the slow degradation of relatively low methane-yielding cellulosic materials. The products resulting from fermentation require an additional transformation before being able to produce methane. It is here that intervene the acetogenes reducing bacteria and the sulfato-reducing bacteria, producing hydrogen sulphide (H₂S). The ultimate phase during which two types of methanogenes bacteria take over: the first ones (acetogenes) reduce methane acetate, CH₄

Table 5.15 Production of biogas from agricultural and horticultural waste

Biogas (ml)	End of 7th day (ml)	End of 15th day (ml)	End of 30th day (ml)	End of 45th day (ml)	End of 60th day (ml)
AW T₁	550.00 (57.51)	720.30 (58.12)	980.20 (59.02)	580.00 (46.20)	550.50 (35.22)
AW T₂	680.30 (57.33)	700.20 (58.35)	950.30 (58.89)	620.30 (45.70)	580.00 (36.24)
HW T₁	500.60 (51.74)	580.30 (54.30)	500.20 (57.56)	420.00 (45.08)	380.30 (34.12)
HW T₂	490.20 (51.52)	600.20 (53.96)	580.00 (54.13)	500.30 (44.96)	450.00 (35.15)

AW- Agricultural waste

AW-T₁-M₁-C₁- Biogas production without pretreatment

AW-T₂-M₁-C₂- Biogas production with pretreatment

HW- Horticultural waste

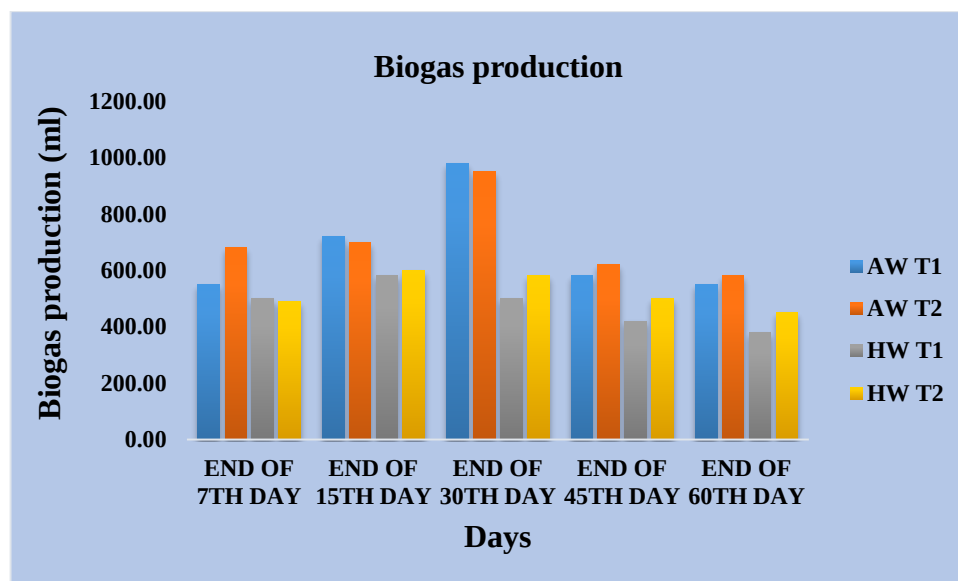
HW-T₁-M₁-C₁- Biogas production without pretreatment

HW-T₂-M₁-C₂- Biogas production with pretreatment

Values are mean of three replications

*The table values with in the brackets indicate the composition of methane per cent of different treatments.

Fig 5.13 Production of biogas from agricultural and horticultural waste



AW- Agricultural waste

AW-T₁-M₁-C₁- Biogas production without pretreatment

AW-T₂-M₁-C₂- Biogas production with pretreatment

HW- Horticultural waste

HW-T₁-M₁-C₁- Biogas production without pretreatment

HW-T₂-M₁-C₂- Biogas production with pretreatment

Values are mean of three replications

and bicarbonate. The second ones reduce methane bicarbonate. Rises in the methane content of biogas as a result of a decrease in the bioreactor temperature. The increase in the quality of biogas is attributed to the raised solubility of carbon dioxide at the lower temperature cycle.

The above results were similar to that of Vikrant and Shekar. (2013) who studied on the anaerobic digestion of horticulture waste for production of biogas with combination of the mixed inoculum was used for biogas production at 37 °C in laboratory (small scale) reactor and results were obtained as in between 10 to 150 ml during the process of anaerobic digestion.

In the above result methane (CH₄) in the four treatments was similar to that of Ziganshin *et al.* (2013) who conducted an experiment was anaerobic digestion in laboratory scale biogas reactors fed with different agricultural waste materials and obtained the results of methane (CH₄) (57.50, 51.70 and 44.20 % in different biogas reactors).

4.4.2 Bio Ethanol Production from Agricultural and Horticultural Waste Enriched With and Without Cellulose Degrading Bacteria

The results of ethanol estimation during 5 days of duration revealed that, at 1st day highest bioethanol content was recorded in AW-T₂ (Bioethanol production with pretreatment) (0.023 g l⁻¹) and HW-T₂ (Bioethanol production with pretreatment) whereas lowest was recorded in HW-T₁ (Bioethanol production without pretreatment) (0.016 g l⁻¹). At 2nd and 3rd day highest bioethanol content was recorded in HW-T₁ (Bioethanol production without pretreatment) (0.056 g l⁻¹) and lowest in AW-T₁ (Bioethanol production without pretreatment) (0.029 and 0.033 g l⁻¹). At 4th day highest bioethanol content in HW-T₁ (Bioethanol production without pretreatment) (0.058 g l⁻¹) followed by lowest in AW-T₂ (Bioethanol production with pretreatment) (0.041 g l⁻¹). At 5th day the highest bioethanol content was recorded in HW-T₁ (Bioethanol production without pretreatment) (0.050 g l⁻¹) and lowest in AW-T₁ (Bioethanol production without pretreatment) (0.034 g l⁻¹). Gradual increase in bioethanol content from 1-4th day was observed among the treatments AW-T₁ (Bioethanol production without pretreatment), AW-T₂ (Bioethanol production with pretreatment) and HW-T₁ (Bioethanol production without pretreatment). Whereas the increase in bioethanol content from 1-3rd day observed in HW-T₂ (Bioethanol production with pretreatment) (Table 5.16). In addition, Horticultural waste hydrolysate seemed to have a slightly positive effect in ethanol

production as evidenced by the higher specific ethanol productivity. This is probably owing to the presence of the hydrolysis by-product, such as acetic acid, which is known to enhance the fermentation rate at a low concentration. Horticultural waste is a potential feedstock for both biogas and bioethanol production.

In the above results the bioethanol evolved from the AW-T₁ (Bioethanol production without pretreatment) (0.033 g l⁻¹, 0.045 g l⁻¹ and 0.034 g l⁻¹) and AW-T₂ (Bioethanol production with pretreatment) (0.042 g l⁻¹, 0.040 g l⁻¹, 0.041 g l⁻¹ and 0.036 g l⁻¹) was more when compared to the observation of Bondesson *et al.* (2013) who conducted an experiment on production of bioethanol from lignocellulose enzymatic hydrolysis, which produces fermentable sugars from carbohydrates present in the corn stover and they obtained the results of ethanol as 0.03 g l⁻¹, 0.09 g l⁻¹ and 1.19 g l⁻¹ in different silage substrates.

In the above results the bioethanol evolved from the two treatments (HW-T₁ (Bioethanol production without pretreatment) and HW-T₂ (Bioethanol production with pretreatment)) was less when compared to the observation of Ajay *et al.* (2014) who conducted an experiment with simultaneous saccharification and fermentation of banana peels to ethanol at different temperatures (20 to 50 °C). They obtained the results of ethanol as 0.638 %, 0.891 %, 2.377 %, 3.032 % and 4.800 % in different days during fermentation process.

Table 5.16. Bioethanol production from agricultural and horticultural waste

Bioethanol (g l⁻¹)	1 Day (g l⁻¹)	2 Day (g l⁻¹)	3 Day (g l⁻¹)	4 Day (g l⁻¹)	5 Day (g l⁻¹)
AW T₁	0.017	0.029 (0.012)	0.033 (0.004)	0.045 (0.012)	0.034 (0.011)
AW T₂	0.023	0.042 (0.019)	0.040 (0.002)	0.041 (0.001)	0.036 (0.005)
HW T₁	0.016	0.051 (0.035)	0.056 (0.005)	0.058 (0.002)	0.050 (0.008)
HW T₂	0.023	0.046 (0.023)	0.055 (0.009)	0.052 (0.003)	0.047 (0.005)

AW- Agricultural waste

AW-T₁-M₁-C₁- Bioethanol production without pretreatment

AW-T₂-M₁-C₂- Bioethanol production with pretreatment

HW- Horticultural waste

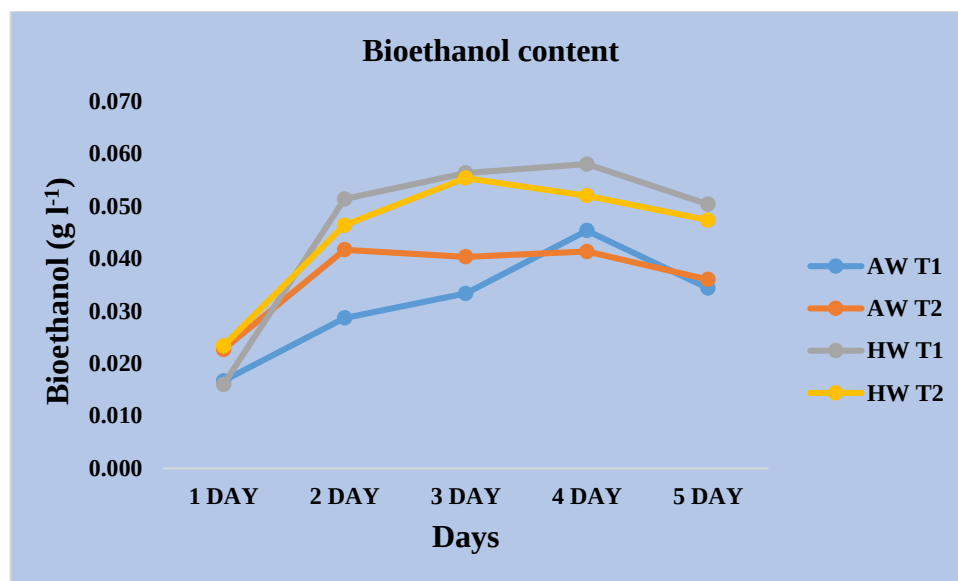
HW-T₁-M₁-C₁- Bioethanol production without pretreatment

HW-T₂-M₁-C₂- Bioethanol production with pretreatment

Values are mean of three replications

*The table values with in the brackets indicate the difference between the values of adjacent days

Fig: 5.14 Bioethanol production from agricultural and horticultural waste



AW- Agricultural waste

AW-T₁-M₁-C₁- Bioethanol production without pretreatment

AW-T₂-M₁-C₂- Bioethanol production with pretreatment

HW- Horticultural waste

HW-T₁-M₁-C₁- Bioethanol production without pretreatment

HW-T₂-M₁-C₂- Bioethanol production with pretreatment

Chapter V

SUMMARY AND CONCLUSIONS

The experiment entitled **“AEROBIC AND ANAEROBIC DIGESTION OF AGRICULTURAL WASTE FOLLOWED BY VERMICOMPOSTING AND ENRICHMENT”** was conducted during 2015-16 at Department of Agricultural Microbiology and Bioenergy, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad.

Samples were collected from different sources i.e. Student farm, farmer's fields, forest soil, horse dung dump, domestic kitchen waste, municipal solid waste, sewage water and cow dung. Total seventy isolates were showed halo zones on cellulose congoed agar media. Among these Thirty isolates showed efficient cellulose degrading cultures on cellulose congoed agar medium. They were further tested qualitatively and quantitatively for cellulase activity. Among thirty isolates, four bacterial isolates were having maximum cellulase activity. Finally these four isolates were (CDB-10, CDB-30, CDB-34 and CDB-36) selected for pretreatment of agricultural (Maize straw) and horticultural waste (Banana fruit waste) to prepare compost, vermicompost under aerobic condition, bioethanol and biogas production under anaerobic condition after enriching the substrates with cellulose degrading cultures.

Biological pretreatment with complex microbial agents proved to be an efficient method to improve biodegradability to enhance composting, vermicomposting and biogas production of agricultural waste and horticultural waste. Compared to untreated controls the pretreated agricultural and horticultural waste yielded higher manurial value and given more biogas production. The enhanced biogas production was attributed to the improved biodegradability of the straw and fruit waste as indicated by increased TS and VS reductions and a shortened digestion time. Cow dung along with other agricultural waste and horticultural waste were used for the biogas production and the same substrates were also used for compost, vermicompost making and alcohol production in lab scale. Along with the estimation of biogas production different parameters like Total Solids (TS %), Total Volatile Solids (TVS %), Volatile Fatty Acids (VFA), pH, Nitrogen (N %), Phosphorous (P %), Potassium (K %), Organic carbon, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), cellulose %, microbial population count, methane and bioethanol percentage were estimated.

Total Solids (TS %) in the substrate was significantly more in T₁ (Composting without pretreatment) 19.46 %. By the end of the process of pretreatment Total Solids (TS %) in the substrate was comparatively least recorded in T₆ (Biogas production with pretreatment) 10.43 %. By the end of the process of aerobic composting and anaerobic digestion, the Total Solids (TS %) was lowest recorded in T₅ (Biogas production without pretreatment) 7.05 %. Total Volatile Solids (TVS %) in the substrate was significantly more in T₂ (Composting with pretreatment) 80.46 %. By the end of the process of pretreatment Total Volatile Solids (TVS %) in the substrate was comparatively least recorded in T₁ (Composting without pretreatment) 62.38 %. By the end of the process of aerobic composting and anaerobic digestion, the Total Volatile Solids (TVS %) was lowest in T₅ (Biogas production without pretreatment) 45.29 %. Volatile Fatty Acids (VFA) concentration in the substrate was significantly more in T₁ (Composting without pretreatment) 0.95 g l⁻¹. By the end of the process of pretreatment Total Volatile Solids (TVS %) in the substrate was comparatively least recorded in T₃ (Vermicomposting without pretreatment) 1.15 g l⁻¹. By the end of the process aerobic composting and anaerobic digestion, the Volatile Fatty Acids (VFA) was lowest in T₄ (Vermicomposting with pretreatment) 0.38 g l⁻¹.

pH in the substrate was significantly more in T₃ (Vermicomposting without pretreatment) 8.25. By the end of the process of pretreatment pH in the substrate was comparatively least recorded in T₅ (Biogas production without pretreatment) 6.38. By the end of the process aerobic composting and anaerobic digestion, the pH was lowest in T₄ (Vermicomposting with pretreatment) 7.21.

Nitrogen % in the substrate was significantly more in T₄ (Vermicomposting with pretreatment) 1.84 %. By the end of the process aerobic composting, and anaerobic digestion, the N % was highest in T₃ (Vermicomposting without pretreatment) 2.00 %. P % was highest in T₅ (Biogas production without pretreatment) 1.85 %. K % was highest in T₅ (Biogas production without pretreatment) 1.75 %.

Organic carbon % in the substrate was significantly more in T₆ (Biogas production with pretreatment) 45.60 %. By the end of the process aerobic composting, and anaerobic digestion, the Organic carbon % was lowest in T₄ (Vermicomposting with pretreatment) 22.52 %. BOD in the substrate was significantly more in T₄ (Vermicomposting with pretreatment) 98.30 mg l⁻¹. By the end of the process of pretreatment BOD in the substrate was comparatively least recorded in T₁ (Composting without pretreatment) 95.90 mg l⁻¹. By the end of the process aerobic composting and anaerobic digestion, the BOD was

lowest in T₁ (Composting without pretreatment) 45.80 mg l⁻¹. COD in the substrate was significantly more in T₄ (Vermicomposting with pretreatment) 120.40 g l⁻¹. By the end of the process of pretreatment COD in the substrate was comparatively least recorded in T₄ (Vermicomposting with pretreatment) 97.23 g l⁻¹. By the end of the process aerobic composting and anaerobic digestion, the COD was lowest in T₅ (Biogas production without pretreatment) 59.46 g l⁻¹.

Cellulose % in the substrate was significantly more in T₂ (Composting with pretreatment) 27.58 %. By the end of the process of pretreatment cellulose % in the substrate was comparatively least recorded in T₆ (Biogas production with pretreatment) 15.85 %. By the end of the process aerobic composting and anaerobic digestion, the cellulose % was lowest in T₆ (Biogas production with pretreatment) 12.85 %.

Population of bacteria, fungi and actinomycetes in the substrate was significantly more in T₁ (Composting without pretreatment) 9.9 x 10⁶ CFU g⁻¹, T₂ (Composting with pretreatment) 4.4 x 10⁴ CFU g⁻¹ and T₁ (Composting without pretreatment) 9.8 x 10⁴ CFU g⁻¹. By the end of the process aerobic composting and anaerobic digestion, the population of bacteria, fungi and actinomycetes was lowest in T₄ (Vermicomposting with pretreatment), T₆ (Biogas production with pretreatment) 3.5 x 10⁶ CFU g⁻¹, T₁ (Composting without pretreatment) 2.1 x 10⁴ CFU g⁻¹ and T₅ (Biogas production without pretreatment) 4 x 10⁴ CFU g⁻¹.

Similarly in horticultural waste Total Solids (TS %) in the substrate was significantly more in T₆ (Biogas production with pretreatment) 13.25 %. By the end of the process of pretreatment Total Solids (TS %) in the substrate was comparatively least recorded in T₆ (Biogas production with pretreatment) 13.49 %. By the end of the process aerobic composting and anaerobic digestion, the Total Solids (TS %) was lowest recorded in T₅ (Biogas production without pretreatment) 6.99 %. Total Volatile Solids (TVS %) in the substrate was significantly more in T₆ (Biogas production with pretreatment) 86.35 %. By the end of the process of pretreatment Total Volatile Solids (TVS %) in the substrate was comparatively least recorded in T₄ (Vermicomposting with pretreatment) 79.89 %. By the end of the process aerobic composting and anaerobic digestion, the Total Volatile Solids (TVS %) was lowest in T₅ (Biogas production without pretreatment) 53.41 %. Volatile Fatty Acids (VFA) concentration in the substrate was significantly more in T₃ (Vermicomposting without pretreatment) 1.12 g l⁻¹. By the end of the process of pretreatment total volatile solids % in the substrate was comparatively least recorded in T₃ (Vermicomposting without pretreatment) 0.74 g l⁻¹. By the end of the process aerobic

composting and anaerobic digestion, the Volatile Fatty Acids (VFA) was lowest in T₃ (Vermicomposting without pretreatment) 0.60 g l⁻¹.

pH in the substrate was significantly more in T₄ (Vermicomposting with pretreatment) 8.34. By the end of the process of pretreatment pH in the substrate was comparatively least recorded in T₅ (Biogas production without pretreatment) 6.12. By the end of the process aerobic composting and anaerobic digestion, the pH was lowest in T₄ (Vermicomposting with pretreatment) 7.33.

Nitrogen % in the substrate was significantly more in T₄ (Vermicomposting with pretreatment) 1.84 %. By the end of the process aerobic composting, and anaerobic digestion, the N % was highest in T₄ (Vermicomposting with pretreatment) 2.80 %. P % was highest in T₅ (Biogas production without production) 1.90 %. K % was highest in T₃ (Vermicomposting without pretreatment) and T₅ (Biogas production without pretreatment) 1.58 %.

Organic carbon % in the substrate was significantly more in T₆ (Biogas production with pretreatment) 40.90 %. By the end of the process aerobic composting and anaerobic digestion, the lowest Organic carbon % was recorded in T₂ (Composting with pretreatment) 23.2 %. BOD in the substrate was significantly more in T₃ (Vermicomposting without pretreatment) 100.20 mg l⁻¹. By the end of the process of pretreatment BOD in the substrate was comparatively least recorded in T₁ (Composting without pretreatment) 80.26 mg l⁻¹. By the end of the process aerobic composting and anaerobic digestion, the BOD was lowest in T₅ (Biogas production without pretreatment) 49.45 mg l⁻¹. COD in the substrate was significantly more in T₄ (Vermicomposting with pretreatment) 110.60 g l⁻¹. By the end of the process of pretreatment COD in the substrate was comparatively least recorded in T₄ (Vermicomposting with pretreatment) 96.70 g l⁻¹. By the end of the process aerobic composting and anaerobic digestion, the COD was lowest in T₅ (Biogas production without pretreatment) 59.39 g l⁻¹.

Cellulose % in the substrate was significantly more in T₂ (Composting with pretreatment) 25.38 per cent. By the end of the process of pretreatment cellulose % in the substrate was comparatively least recorded in T₆ (Biogas production with pretreatment) 15.89 %. By the end of the process aerobic composting and anaerobic digestion, the cellulose % was lowest in T₆ (Biogas production with pretreatment) 14.03 %.

Population of bacteria, fungi and actinomycetes in the substrate was significantly more in T₁ (Composting without pretreatment) 10 x 10⁶ CFU g⁻¹, T₁ (Composting without

pretreatment) 9×10^4 CFU g⁻¹ and T₅ (Biogas production without pretreatment) 5.1×10^4 CFU g⁻¹. By the end of the process of pretreatment population dynamics of bacteria, fungi and actinomycetes in the substrate was comparatively least recorded in T₂ (Composting with pretreatment) 6.3×10^6 CFU g l⁻¹, T₁ (Composting without pretreatment) 8×10^4 CFU g⁻¹ and T₆ (Biogas production with pretreatment) 4.5×10^4 CFU g l⁻¹. By the end of the process aerobic composting and anaerobic digestion, the population of bacteria, fungi and actinomycetes was lowest in T₂ (Composting with pretreatment) 5.7×10^6 CFU g⁻¹, T₁ (Composting without pretreatment) 7×10^4 CFU g⁻¹ and T₆ (Biogas production with pretreatment) 3.7×10^4 CFU g⁻¹

Biogas production was studied in the lab with four treatments and three replications each and with 250 grams cowdung, 500 grams substrate and 1000 ml water (1:2:5) in three litres glass bottles.

Significantly highest gas production was observed in AW-T₂ (Biogas production with pretreatment) 3531.1 ml compared to AW-T₁ (Biogas production without pretreatment) 3381 ml, HW-T₂ (Biogas production with pretreatment) 2620.7 ml and less in HW-T₁ (Biogas production without pretreatment) 2381.4 ml.

Significantly highest ethanol production was observed in HW-T₂ (Biogas production with pretreatment) 0.223 g l⁻¹ compared to HW-T₁ (Biogas production without pretreatment) 0.231 g l⁻¹, AW-T₂ (Biogas production with pretreatment) 0.182 g l⁻¹ and less in AW-T₁ (Biogas production without pretreatment) 0.158 g l⁻¹.

In the present study, results revealed that agricultural and horticultural wastes pretreatment with efficient microbes helped aerobic composting, vermicomposting, and bioethanol and biogas production under anaerobic condition. By the pretreatment of agricultural waste, horticultural waste were easily degraded by enriched cultures and their enzyme activities. In the present study vermicompost with microbial pretreatment enhanced degradation and nutrient values compared to regular composting methods. Compared to aerobic composting, vermicomposting and anaerobic digestion showed to be better, more useful for biogas production and manurial value.

However, all the combinations were good in terms of their manurial value as the total solids, total volatile solids percentage will be higher in horticultural waste (pretreated one rather than without pretreated one) compared to agricultural waste. The N, P and organic carbon % increased in all the treatments of horticultural waste compared to agricultural waste. Considering the characteristics of the high moisture solid waste of agricultural and

horticultural waste, anaerobic digestion represents a feasible and effective method to convert the waste to biogas fuel. The agricultural waste was found to be the best in biogas production as compared to horticultural waste. Horticultural waste was comparatively better in terms of N, P, K and organic carbon %.

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APPENDIX I

Composition of different growth media/reagents/indicators used

1. Cellulose Congo Red Agar Medium

Carboxymethyle cellulose	1.88 g
Potassium hydrogen phosphate (K ₂ HPO ₄)	0.5 g
Potassium dihydrogen phosphate (KH ₂ PO ₄)	0.7 g

Ammonium sulphate (NH ₄) ₂ SO ₄	1.0 g
Magnesium sulphate (MgSO ₄ . 7H ₂ O)	0.1 g
Congored	0.20 g
Agar	10 g
pH	7.0 ± 0.2
2. Glucose Broth	
Peptone	5.00 g
Beef extract	3.00 g
Glucose	5.00 g
Distilled water	1000 ml
Bromo Cresol Purple (BCP) Solution	15 ml
pH	7.0 ± 0.2
3. Glucose Peptone Agar	
Glucose	5.00 g
Peptone	10.00 g
Agar	15.00 g
Distilled water	1000 ml
pH	7.0 ± 0.2
4. Hofer's Alkaline Medium	
Yeast extract	1.00 g
Mannitol	10.00 g
Dipotassium hydrogen phosphate	0.50 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.10 g
Thymol blue	0.016 g
Distilled water	1000 ml
pH	11.0
5. Lactose Agar Medium	
Lactose	10.00 g
Yeast extract	1.00 g
Dipotassium hydrogen phosphate	0.50 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.10 g
Calcium carbonate	3.00 g
Agar	20.00 g
Distilled water	1000 ml
pH	7.0
6. MR – VP broth	
Buffered peptone	7.00 g
Dextrose	5.00 g
Dipotassium phosphate	5.00 g
Distilled water	1000 ml
pH	6.9 ± 0.2
7. Nutrient Agar	
Peptone	5.00 g
Beef extract	3.00 g

Sodium chloride	5.00 g
Agar	18.00 g
Distilled water	1000 ml
pH	6.8 – 7.2

8. Nutrient Gelatin Agar

Peptone	5.00 g
Beef extract	3.00 g
Gelatin	120.00 g
Distilled water	1000 ml
pH	6.8 – 7.0

9. Pikovskaya's Agar

Yeast extract	0.50 g
Dextrose	10.00 g
Calcium phosphate	5.00 g
Ammonium sulphate	0.50 g
Potassium chloride	0.20 g
Manganese sulphate	0.0001 g
Magnesium Sulphate	0.10 g
Ferrous sulphate	0.0001 g
Agar	20.00 g
Distilled water	1000 ml
pH	7.0

10. Plate Count Agar

Casein enzymic hydrolysate	5.00 g
Yeast extract	2.00 g
Dextrose	1.00 g
Agar	0.00 g
Distilled water	1000 ml
pH	7.0 ± 0.2

11. Potato Dextrose Agar

Potato infusion	200.00 g
Dextrose	20.00 g
Agar	18.00 g
Distilled water	1000 ml
pH	5.6 ± 0.2

12. Simmons Citrate Agar

Magnesium Sulphate	0.20 g
Ammonium dihydrogen phosphate	1.00 g
Dipotassium phosphate	1.00 g
Sodium citrate	2.00 g
Sodium chloride	5.00 g
Bromothymol blue	0.08 g
Agar	18.00 g
Distilled water	1000 ml
pH	6.8 ± 0.1

13. Starch Casein Agar

Starch	10.00 g
Casein powder	1.00 g
Agar	18.00 g
Distilled water	1000 ml
pH	7.2 ± 0.2

14. Urea Broth

Yeast extract	0.10 g
Potassium dihydrogen phosphate	9.10 g
Disodium hydrogen phosphate	9.50 g
Urea	20.00 g
Phenol red	0.01 g
Distilled water	1000 ml
pH	6.8

15. Yeast Extract Mannitol Congored Agar

Yeast extract	1.00 g
Mannitol	10.00 g
Dipotassium hydrogen phosphate	0.50 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.10 g
Congo red	0.025 g
Agar	20.00 g
Distilled water	1000 ml
pH	6.8 – 7.0

16. Yeast Extract Mannitol Broth

Yeast extract	1.00 g
Mannitol	10.00 g
Dipotassium hydrogen phosphate	0.50 g
Magnesium Sulphate	0.20 g
Sodium chloride	0.10 g
Calcium carbonate	1.00 g
Distilled water	1000 ml
pH	6.8 – 7.0

16. Pseudomonas Agar (For Fluorescein)

Pancreatic digest of casein	10 g
Peptide digest of animal tissue	10 g
Anhydrous dibasic potassium phosphate	1.50 g
Magnesium sulphate	1.50 g
Agar agar	15.0 g
pH	7.0

17. Nitrate Broth

Peptone	5.0 g
Beef extract	3.0 g
Potassium nitrate	5.0 g
Water	1000 ml
pH	6.8-7.0

18. King's B agar medium

Peptone	16.0 g
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K ₂ HPO ₄	1.6 g
MgSO ₄	1.6 g
Glycerol	10.0 g
Agar	18.00 g
Distilled water	1000 ml

19. Gram Stain Solutions

a) Crystal violet solution

Crystal violet	10.0 g
Ammonium oxalate	4.0 g
Ethanol	100 ml
Distilled water	1000 ml

b) Gram's Iodine solution

Iodine	1.0 g
Potassium iodide	2.0 g
Ethanol	25 ml
Distilled water	100 ml

c) Counter stain

2.5% safranin in ethanol	10 ml
Distilled water	100 ml

d) Ethyl alcohol (Decolouriser)

Ethanol	95 ml
Distilled water	5 ml

20. Salkowski Reagent

35% perchloric acid	50 ml
0.5M FeCl ₃	1 ml

APPENDIX 2

Reagents used for chemical analysis of substrates used for composting,
vermicomposting, bioethanol and biogas production

1. pH

Standard buffer solution: prepare buffer solution of pH 4.0, 7.0 and 9.2 in pure water, or of other pH value in the acidic and alkaline range. Alternatively, dissolve one commercially available buffer tablet in dissolved water and make the volume of 1000 ml. Prepare a fresh buffer solution after every few days as these solutions do not keep for long. A 0.05 M solution of AR grade potassium hydrogen phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$, mol. Wt. 204.22) gives a pH of 4.00 at 25°C and this can also be used as a standard buffer. For this prepare a stock solution of 0.25 M by dissolving 51.04 gm of the pure dry salt in warm water and making volume to 1000 ml. three to four drops of toluene are added to prevent the growth of mould. A 10 ml portion of this solution diluted to 50 ml with water gives 0.05 M solution. Similarly, a solution containing 19.45 ml of 0.2 M Na_2HPO_4 and 0.55 ml of 0.1 M citric acid gives a buffer of pH 8.0.

2. Nitrogen

Reagents:-

- 1) 0.32 % KMnO_4 : Dissolve 3.2 g of KMnO_4 in water and make to one litre.
- 2) 2.5 % NaOH : Dissolve 2.5 g of sodium hydroxide pellet in water and make to one litre.
- 3) 2 % Boric acid: Dissolve 20 g of boric acid powder in warm water by stirring and dilute to one litre.
- 4) Mixed indicator: Dissolve 0.066 g of methyl red and 0.099 g of bromocresol green in 100 ml of ethyl alcohol. Add 20 ml of this indicator to each litre of 2 % boric acid solution. Adjust the pH to 4.5 with dil. HCl or dil. NaOH .
- 5) 0.1 N Potassium hydrogen phthalate: Dissolve 20.422 g of the salt in water and dilute to one litre. This is a primary standard and does not require standardization.
- 6) 0.1N NaOH : Dissolve 4 g of NaOH in water and dilute so one litre. Standardize it against 0.1 M Potassium hydrogen phthalate solution.
- 7) 0.02 N H_2SO_4 : Prepare approximately 0.1 N H_2SO_4 by adding 2.8 ml of cone. H_2SO_4 to about 990 ml of distilled water. From this, prepare 0.02 N H_2SO_4 by adding diluting a suitable volume five times with distilled water. Standardize it against 0.1 N NaOH solution.

3. Phosphorus

Diacid digestion: It is carried out using 5:1 of HNO_3 : HClO_4 if the sample is high in fats / oils, pre-digestion using 25 ml HNO_3 / g sample is recommended to avoid explosion.

Triacid digestion: It is carried out using a mixture of HNO_3 : H_2SO_4 : HClO_4 in the ratio of 9 : 4 : 1.

Reagents:-

1. Ammonium molybdate – ammonium vanadate in HNO_3 (Barton's reagent)
 - A. Dissolve 22.5 g of ammonium molybdate in 400 ml of warm distilled water.
 - B. Also dissolve 1.25 g of ammonium metavanadate 150 ml of boiling water.
 - C. Add the two solutions and cool it to room temperature.
 - D. Add 250 ml of concentrated nitric acid (HNO_3) and dilute to 1 L.
2. Standard P solution: Dissolve 0.2195 g of analytical grade KH_2PO_4 and dilute to 1 L.

4. Potassium

Preparation of working standards: Dissolve 1.907 g of AR grade KCl in 1 litre of distilled water. This gives 1000 ppm K. From this different working standards are prepared. Dilute 0, 1, 2, 3, 4 and 5 ml of the stock solution to 100 ml to obtain 0, 10, 20, 30, 40 and 50 ppm K working standards.

5. Organic carbon

Reagents:-

- 1) 1 N Potassium dichromate: Dissolve 49.04 g of AR grade $\text{K}_2\text{Cr}_2\text{O}_7$ in about 500 ml of distilled water and make the volume to one litre.
- 2) Cone. Sulphuric acid
- 3) 0.5 N Ferrous ammonium sulphate: Dissolve 196 g of ferrous ammonium sulphate in distilled water, add 20 ml of conc. H_2SO_4 and make up volume to one litre. The ferrous ammonium sulphate should be from fresh lot and in light green colour. Yellowing of the salt indicates its oxidation.
- 4) Diphenylamine indicator: Dissolve 0.5 g of the dye in a mixture of 20 ml of distilled water and 100 ml of cone. H_2SO_4 .
- 5) Orthophosphoric acid (85 %) or sodium fluoride.

6. Biological Oxygen Demand (BOD)

- 1) Manganous sulphate solution: Dissolve 100 g of manganous sulphate in 200 mL of previously boiled distilled water and filter the solution.
- 2) Alkaline potassium iodide solution: Weigh 50 g of potassium iodide and 100 g

of potassium hydroxide. Dissolve the chemicals in 200 mL of previously boiled distilled water.

- 3) Sodium thiosulphate solution (0.025 N): Take 6.205 g of sodium thiosulphate, dissolve it in previously boiled distilled water and make up the volume to 1 L. Add a pallet of sodium hydroxide as a preservative. Keep the solution in coloured bottle.
- 4) Starch indicator: Dissolve 1 g of starch in 100 mL of warm distilled water and add few drops of toluene or formaldehyde as preservative.
- 5) BOD-free water: Pass the deionized glass distilled water through a column of activated carbon and redistill it.
- 6) Phosphate buffer solution: Dissolve 42.53 of potassium dihydrogen phosphate in 700 ml BOD free water and add 8.8 g NaOH. Adjust the pH 7.2. Add 2g ammonium sulphate and dilute to 1 litre with BOD free water.
- 7) Magnesium sulphate solution: Dissolve 82.5 g of magnesium sulphate in BOD free distilled water to prepare 1 L of solution.
- 8) Calcium chloride solution: Dissolve 275 g of anhydrous calcium chloride in BOD free distilled water to prepare 1 L solution.
- 9) Ferric chloride solution: Dissolve 0.25 g of ferric chloride in 1 L of BOD free distilled water.
- 10) Sulphuric acid (N): Add 2.8 mL of concentrated sulphuric acid to 100 mL of BOD free distilled water.
- 11) Sodium hydroxide solution (1N): Add 4 g of sodium hydroxide in BOD free distilled water and make the volume 100 mL.

7. Chemical Oxygen Demand (COD)

- 1) Potassium dichromate solution (0.25 N): Dissolve 12.259 g of AR grade potassium dichromate previously dried at 103⁰ C in distilled water. Add about 120 mg sulphuric acid to this and dilute to 1 L.
- 2) Dry powder of silver sulphate
- 3) Dry powder of mercuric sulphate
- 4) Concentrated sulphuric acid
- 5) Ferrion indicator solution: Dissolve 0.695 g of ferrous sulphate and 1.485 g of 1, 10- phenanthroline in distilled water to make 100 mL of indicator solution.
- 6) Standard ferrous ammonium sulphate solution (0.25 N): Dissolve 98 g of ferrous ammonium sulphate in distilled water add 20 mL of sulphuric acid, cool and dilute to 1 L further by adding distilled water.

8. Ethanol estimation:

Reagent:-

Potassium dichromate solution: take 33.76 g of $\text{K}_2\text{Cr}_2\text{O}_7$ in 400 ml of distilled water with 325 ml of H_2SO_4 volume was raised to 1 lit.